

The future for UK university research?

SOME time ago, the British evolved the dictum that ships should not be spoiled for a ha'porth of tar. Now they seem to think that ships can be mended with the same material and at much the same cost. Such, at least, is the response so far to the mounting evidence of the 1970s that the British university system is in danger of collapse or, perhaps worse, of lapsing into mediocrity. There is to be an examination of the mechanism for supporting academic research by a committee whose chairman is Sir Alec Merrison (Vice-Chancellor of the University of Bristol) and among whose members representatives of the research councils and the University Grants Committee are conspicuous. The immediate objective is to make recommendations about the system by means of which the two sets of bodies separately provide funds for university research. The committee hopes to have something to say by the end of the year, but that is unlikely to be more than a judicious restatement of what people in university laboratories already know — that the dual support system, regarded as a monument to the British sense of fair play in earlier decades, has now quite broken down.

British universities are not of course alone in their adversity. In most industrialised countries, governments have found it necessary to call a halt to the rapid growth of public support for university teaching and research that began, in most places, in the early 1960s. Strains have, for example, been apparent for some years in the United States, where the competition for funds by university scientists has become more fierce and where the grant-making agencies themselves have been forced to make sometimes radical reappraisals of their policies. (Is peer review as equitable as it seems, and conducive the most effective use of funds? Would block-grant support for outstanding institutions yield greater benefits and, if so, would Congress ever agree?) The British problem, however, is made much more difficult because the virtual stagnation of public budgets for the past several years has been imposed on a university system so delicately balanced between conflicting interests that it cannot respond quickly to the need for change. As the ecologists were once fond of saying, everything depends on everything else.

The dual support system is such a case. The theory is simple, even admirable. The University Grants Committee provides all the universities on its books with the facilities needed to teach students, graduate students included. The UGC grant to the universities also acknowledges the requirement of the standard contract with academics that half of a person's time should be spent on research. The research councils — the Science Research Council especially — provide extra support for individual project research and for facilities which are in principle open to all universities. (Thus the Science Research Council pays the British contribution to the European Centre for Nuclear Research at Geneva.) The research councils are also the chief source of stipends for graduate students. So, the theory has it, all academics are enabled to teach well, and to conduct research. But outstanding people can compete for extra support on the merits of their proposals.

The gulf between theory and practice has quickly widened in the past few years. That part of the UGC support for universities called the Equipment Grant (now exceeding £80 million a year), which is distributed among universities separately from recurrent costs, is often too little for university departments to meet the costs of servicing essential equipment, electron microscopes or

computers for example. The general shortage of funds also requires that many academics, short of technical assistance, have to work as their own repairmen. Direct support for research is at the same time increasingly under strain. Probably it is still true that outstanding projects win support from one or other of the research councils, but there is an alarming tendency towards cheeseparing that often seems (to grant recipients) calculated to ensure that even the best projects will not quite succeed. Less than outstanding but still good projects are now at risk, as are the people employed for years on soft money (from whatever source), who must be now almost as much at risk of unemployment in mid-career as are professional footballers.

All this is quickly changing the pattern and helping further to erode the spirit of much university research. The number of university departments which lack externally funded research projects is growing, further increasing the pressure on UGC funds. The supposition that students will be taught in places where research is active is too often false for comfort. The most serious threat to the future of British university research is that young men and women with outstanding projects in their minds can no longer look confidently for niches within even sympathetic university departments where a research team can grow up. The demographic log-jam among tenured university teachers, more than half of whom are still younger than forty, is largely responsible. The shadow cast by these developments on the future is long and getting darker.

Unhappily for the Merrison committee, these are problems which can be more easily described than solved. The UGC's support of university research rests on the assumption that all academics are equally free to win reputations for themselves in research. By extension, all universities are supposed equal and are thus confirmed in their proper jealousy of their independence. Support from the research councils is, on the other hand, openly and properly discriminatory; the best projects, wherever they come from, attract support. An application from the University of the Sticks has, in theory, as good a chance as one from Oxbridge. Practice is, however, somewhat different. Willy-nilly, successful researchers find their way to places where research is already strong and, once there, attract further support for their departments' research. The lists show that Oxbridge, London and the strong civic universities predominate in the hunt for funds. To ask that such concentrations should not occur is to ask that human nature should be different. The difficulty, for Merrison, is that there can be no reform of the dual-support system that avoids offending against one or other of the principles that all universities are equal and that all academics are free to work where they like (and can find a job).

The temptation, of course, will be to tinker with the system within the limits of the fixed budgets now in prospect. Transferring some of the UGC equipment grant to the research councils, so that each project grant could be accompanied by the full cost of its overheads, would help to ensure that projects were carried out in circumstances where they might succeed, but would be resented (and resisted) by most universities and their teachers. Transferring funds in the opposite direction would be equally offensive. (Constitutionalists will quickly point out that either change would require an Act of Parliament — for which there is no time.) No doubt the committee will also canvass the fashionable notion that industry should directly support more



university research — a dubious precept unlikely to be followed eagerly in the coming recession. Yet something must be done to allow such funds as there are for university research to be concentrated where they can be spent most effectively. The time, in other words, appears to have arrived when the assumption that all universities are equal will have to be openly abandoned. Some will have to draw back from in-house research.

This is where the ructions will begin. The chance is small that a committee with such narrow objectives will discover an acceptable device for working such an important change in the character of the British university system. The ideal would be that there should be incentives for helping the universities less strong in research to work out some other role for themselves, perhaps as specialised institutions or even as liberal arts colleges. The

uniformity of the British university system has been for decades its most obvious weakness. But such a change could be brought about only by a more radical reform of higher education as a whole. Before it knows where it is, the Merrison committee will find itself agonising about the selection of graduate students (now somewhat capricious), transfer of undergraduates between one university and another (still unreasonably difficult), the length of undergraduate courses (most probably too short) and the education of secondary school students (ridiculously narrow). Its problem is both the tip of an iceberg and a can of worms. It will be solved without permanent damage only if the universities themselves seize the initiative and work out a solution for themselves. Their jealousy of their autonomy has been matched in recent years only by their complacency.

Inflation, interest rates and innovation

The United Kingdom in its present condition is likely to be most other nations' illustration of how not to conduct affairs. How can it be, the question will be phrased, that within a year of the election of a government dedicated to deflationary policies, the inflation rate has almost doubled (to 21.8 per cent per annum) and is certain to go still higher? Does it follow that Mrs Margaret Thatcher has misled one of the world's oldest democracies, and should promptly be replaced as Prime Minister? Or that Professor Milton Friedman has misled Mrs Thatcher, and should be stripped of his Nobel Prize? Or is it possible — this is what people elsewhere may ask — that there is some diabolic logic in the British predicament that will in due course make its influence felt elsewhere? There, but for the grace of God, so to speak, go all of us? Or is there another box in the response-frame of this implicit multiple-choice question, something labelled "Other — please specify"?

The intelligent response, of course, is none of the first. Mrs Thatcher is much like other British Prime Ministers — quick, masterful (not synonymous with "masterly") yet unwilling to give hostages to fortune by saying publicly (or even privately) what she hopes for. The innocent Professor Friedman has done nothing worse than to point to an almost arithmetical identity — that (*ceteris paribus*) governments worried about inflation have the remedy in their own hands, by their control of the supply of money, pound notes, dollar bills or what have you. If Professor Friedman were to be robbed of his Nobel Prize simply because, ten years ago, he failed to foresee the subtlety of the ways in which people would counter the stratagems of the deflationary governments they had elected by discovering new ways of raising money or, what is the same thing, buying goods, then it would be proper that other Nobel prizewinners such as Watson and Crick should be on the same plane to the medal-returning ceremony in Stockholm for having failed to guess that reverse transcriptase would be discovered. None of them will make that journey, and rightly.

The question whether we are all destined for the same fate is more tricky. Ten years ago, the Italian economy was widely regarded as a joke in poor taste. The economic miracle of the late 1950s seemed to have vanished as mysteriously as it had arrived. Inflation bobbed around at about 20 per cent a year, a then unprecedented rate for serious industrialised countries. Governments came and went with predictable irregularity. Italy has however managed to survive, largely by means of two separate devices. First, there has been a steady erosion of the strength of social institutions, both modern and ancient. The power of the central government has at the same time steadily declined, sometimes in favour of stronger regional government, sometimes urban terrorism and sometimes both. These are but the predictable consequences of inflation and do not amount to a recipe for survival. Italy has survived in spite of inflation because Italian industry has been able somehow to keep ahead, producing a range of highly saleable commodities which have sold well abroad and

at the same time building on the reputations of Italian engineering contractors for the efficient conduct of major construction projects anywhere in the world. In short, Italy has survived more than a decade of high inflation partly by paying a heavy price and partly by the ingenious exploitation of technology.

In principle the same expedient could be followed in the United Kingdom and, for that matter, by the other potentially sick men of the OECD area, the United States and possibly even Japan. Yet circumstances are now more difficult than when Italy learned how to live with inflation. The price of oil has increased ten times. The result is not merely that prices have increased and that the financial markets are flooded with unused credit, but that the populations of the countries where inflation is highest have not accustomed themselves to the notion that the increased price of oil entails a transfer of wealth to the oil suppliers. Mrs Thatcher was right to insist, last week, that British inflation will not abate until British voters acknowledge that they are individually and collectively impoverished by the increased price of oil.

Unfortunately, in the absence of such realism, even the Italian solution is less accessible. When inflation is high and unlikely quickly to abate, interest rates are bound also to rise. This is what has happened dramatically in the United Kingdom and the United States in the past twelve months. For why should people lend their money either to governments or to those wishing to start new enterprises if they have no expectation that the real value of their money will be returned to them? In principle, of course, the arithmetical rate of interest should not be an absolute brake on innovation. People confident that some new process or product will quickly dominate its rivals can simply allow for the high rate of interest in the prices which they eventually fix for what they have to sell. Reality, alas, is not quite like that. High interest rates mean proportionately greater costs for research, development and the building of new plant. In other words, the real risks of innovation are increased. The dice are loaded against those who might help to solve their countries' problems by technical innovation.

For the United Kingdom and, perhaps later, for the United States, the consequences could be calamitous. (Japan is a different case, whose problems stem not from a lack of productivity or innovation but from the financial problems of entire dependence on imported oil.) That there is a connexion between the soundness of an economy — and in particular its freedom from inflation — and the pace of technical innovation is well attested not merely by what economists say but by the economic statistics of the past ten years. Even in the United States, the rate of growth of industrial productivity has lagged well behind that of the well-founded European states. In the United Kingdom, notoriously, the pace of technical innovation has been a snail's pace. To know that these circumstances can be remedied only if there is a profound change in ordinary people's expectations of prosperity is forbidding. Too many people have a vested interest in not saying what the truth is.

Congress may threaten NIH autonomy

Washington

FEARS for the political independence of the National Institutes of Health have once again come to the surface. The cause of the trouble is a Bill now working its way through the House of Representatives that would require that the several institutes of the NIH are funded individually by Congress.

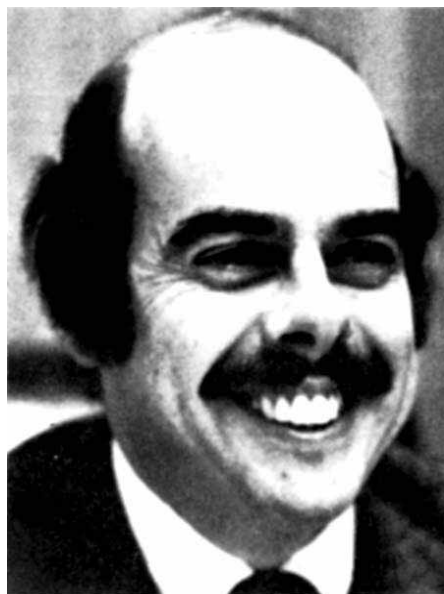
The scientific community is alarmed that the proposed arrangements would enable Congress — or individual congressmen — to attach unworkable provisions to financial legislation. Meanwhile, the administrators of the NIH have drawn the wrath of their political bosses at the Department of Health and Human Services (previously Health, Education and Welfare) by their attempts to head off the legislation in its earlier stages.

At one stage, it is widely reported, the Director of the NIH, Dr Donald Fredrickson, was told by the Secretary of DHHS, Mrs Pat Harris, to "shut up or get out". Dr Fredrickson has stayed put.

Relations between the NIH and its sponsoring department have never been easy. On this occasion, however, the House Bill may seem a lesser evil than a similar piece of legislation introduced in the Senate last year by Senator Edward Kennedy, chairman of the Senate Health and Science Subcommittee.

Kennedy's Bill would make all the institutes of the NIH separately accountable to Congress, but would also have imposed several other unpalatable conditions on the planning of medical research. One of these persists in the version of the Kennedy Bill soon to be considered by the Senate — the proposal that NIH strategy should be determined by a sixteen-member council appointed by the President.

Waxman proposes . . .



The Administration is staunchly opposed to this condition, but appears ready to accept that separate accountability will in future be required of the institutes. This position appears to have been reached only after an acrimonious dispute within the DHHS, where Mrs Harris was at first strongly opposed to some aspects of the House Bill.

The chief consequences of the Bill as it stands will be budgetary. (Two of the eleven institutes of the NIH, the National Cancer Institute and the National Heart, Lung and Blood Institute, are already dealt with separately in Congress.)

Budget strategy will be approved on a three-year basis, with a provision that



. . . Harris disposes

spending may be continued for a fourth year if Congress fails to act. Representative Henry Waxman, chairman of the Health Subcommittee, says however that there is no intention of using the new legislation as a way of cutting individual institute budgets, which will in any case be protected by separate appropriations legislation.

In spite of Mrs Harris's wrath, the NIH have won some concessions from the House of Representatives. A limit on the amount of research grant that could be approved by an individual institute council has been increased from \$50,000 to \$500,000. Moreover, there is a new provision for a fund of \$100 million to be spent on areas considered by the Director of NIH to be especially deserving of support.

NIH officials are still unhappy, however, with the increased role of externally appointed advisory committees in the planning of research strategy, while the extent to which the Secretary of DHHS may delegate responsibilities to the Director is, it appears, to be on the Secretary's say so.

Both the Senate and House bills are likely soon to pass in their respective chambers. In the conference that follows, it should become clear in what corner Mrs Secretary Harris is fighting.

David Dickson

Marine Pollution

Clean the Med

ELEVEN Mediterranean nations signed a treaty in Athens last Saturday (17 May) which commits them to clean up their rivers and sewage discharges, to make the Mediterranean a fit place to swim and fish in again. At present 90 per cent of the sewage from the 120 largest Mediterranean cities is poured into the sea untreated; and Italy, France and Spain sweep out their industrial pollutants through the rivers Po, Rhône and Ebro. Effective control of these land-based sources — the objective of the treaty — will cost some £6 billion, it is estimated. But 100 million tourists a year and a £300 million fishing industry will benefit.

The treaty is the sweetest fruit so far of the United Nations Environment Programme's Regional Seas Programme, which is run by Yugoslav marine scientist Dr Stjepan Keckes. He takes an optimistic view. "The British began talking about cleaning up the Thames 25 years ago. Today, salmon have returned to the Thames. I believe we can make the Mediterranean a much cleaner and safer place by the end of this decade."

The foundation of the treaty is both scientific and political: a network of laboratories around the Mediterranean coasts, many of whose staff have been trained and equipped through the Regional Seas Programme. Keckes has insisted on establishing uniform measurement procedures, and has used national laboratories (rather than a single, international expert team) to ensure the respect of national governments for their data. Keckes has also aimed the programme largely towards the definition of 'water quality objectives' rather than uniform emission standards; this has the advantage that, say, the relatively undeveloped North African coast can initially accept higher-polluting factories simply because there are fewer of them — so development is not penalised. The main burden will in fact fall on Italy, France and Spain.

The treaty will have to be ratified by six nations before it comes into force. Ratification would follow the passing of the necessary national legislation.

Two classes of pollutant are defined in the treaty: 'black list' and 'grey list' substances. Black list substances will be limited to very low emissions, because of their toxicity, persistence, or accumulation

in the food chain. These include mercury, cadmium, used lubricating oils, certain carcinogens and mutagens, and radioactive materials. Grey list substances will be admitted to the Mediterranean through strict licensing agreements which will take into account the ability of the local environment to absorb them. The grey list includes zinc, copper, lead, titanium, crude oils and hydrocarbons, pathogenic micro-organisms, and non-biodegradable detergents.

Atmospheric pollution of the sea is also covered by the treaty. Measurements on the Baltic have indicated that the air, replete with exhaust fumes, may be the principal source of organic lead in surface waters far from the coasts — but the air-sea interchange is very difficult to measure. The treaty has reinstated a deep-sea, air-borne pollutants monitoring programme which had been axed, on French insistence, a year ago. "The countries recognised it was a mistake" said Keckes.

The next step, says Keckes, is to shift the £400,000 a year research programme, from one largely for training and equipping laboratories, to one of specific common research and data handling — which will be a five- or ten-year programme.

Precise environmental quality objectives, emerging from the research programmes will be defined in time for ratification. A meeting in October is expected to set the first: for bathing and shellfish growing waters (largely a matter of micro-organism levels), and for mercury in shellfish.

Robert Walgate

Research posts

Jobs for the Jugende?

WEST German post-doctoral and just pre-doctoral scientists, searching like others the world over for scarce research positions, will shortly find another 175 posts a year open to them — if the federal and regional governments approve.

The Arbeitsgemeinschaft der Grossforschungseinrichtungen (AGF), an association of the 12 major research centres outside the universities, has asked the governments to back a proposal to create 700 new posts over 4 years, each lasting three to five years, at a total cost of about DM90 million (£22 million). This represents little more than an annual 1% addition to the current AGF budget.

Professor Herwig Schopper, president of the AGF, said this week that the proposal was designed to cope with a national population bulge — which is beginning to swell doctoral numbers at a time when most post-doctoral posts are already filled. "We don't want to lose this

talent just because of a freak of demography" he said. The programme fitted well with another — under discussion in the Ministry of Universities — to create more junior university positions over the same period.

In making the proposal, the AGF (whose members include, for example, the high-energy physics laboratory DESY, and the German cancer research centre at Heidelberg, the DKFZ) has taken account of the age profiles of its member laboratories. An official said this week that fewer than one scientist in a hundred currently retires each year from AGF staffs, releasing fewer than 40 posts. Another 30 are freed by scientists moving to industry, but the total of 70 vacancies is far too few to retain a stable age profile in the laboratories.

However, said the official, retirement rates will begin to rise rapidly in five years. By 1992 retirements are expected to reach 7% a year, the equivalent of 280 posts. "So we just have a valley to bridge."

The proposed appointments would be made through the usual recruitment procedures of AGF members, but specified subjects will be preferred. Thus energy research would receive 175 posts; nuclear and subnuclear physics 140 posts; life sciences 140 (with appointments principally to the DKFZ, to the Gesellschaft für Biotechnologische Forschung, and the Gesellschaft für Strahlen und Umweltforschung); information technology 105; materials science 70; aerospace 35; and technology assessment 35.

The Federal science minister, Herr Volker Hauff, has approved the scheme, but it must now go to the cabinet for a decision. And as the regional (Länder) governments support AGF laboratories to 10% of their budgets, the AGF must seek their approval also. Schopper is confident of success, but he does not expect a decision before the federal elections later this year.

Robert Walgate

Innovation

Biotechnology boom at NRDC

BRITISH biotechnology may benefit from the impending change of managing directors at the National Research Development Corporation. Dr James Cain will succeed Dr William Makinson on 1 June, and is already talking of tripling the corporation's spending on biotechnology in the coming three years.

Dr Cain, a 57-year-old biochemist, is at present head of the NRDC's biosciences group, which contributes four-fifths of the corporation's £18 million licence income.

The NRDC's major earners are the cephalosporin antibiotics — developed by Professor E P Abraham of the University of Oxford — which, said Cain last week, have already earned NRDC at least £80 million through licensing agreements set up by the biosciences group since 1959. Pyrethroid insecticides are "already into millions".

Biological projects, based on patents acquired from university and government laboratories, have thus become the NRDC's bread and butter. They also appear to be good business: the cost of developing an innovation from a successful patent to the point at which it can be licensed tends to be less for biological than for hardware projects.

Investment in hardware projects still dominates the NRDC's investment. At the last count, there were 229 active projects in fields such as instrumentation and engineering, compared with only 18 in the biological sciences. The corporation's total investment in biological projects amounts to £1.5 million, a mere tenth of the NRDC's total investment. Of this £600,000 has been committed to genetic engineering. Cain hopes the £1.5 million will be doubled or tripled in the next three years.

Cain says that the corporation has been talking to industry for some time about biotechnical projects, and one involving crop plant development "is being actively followed up", with a decision possible within six months. On the academic side, "people are coming to us with projects once a month", a vast increase on say five years ago. "We've been getting experience of genetic engineering and want to increase our exposure" he said.

Most of the NRDC's projects involve collaboration with industry, but the most natural partner for many joint ventures, the pharmaceutical industry, is so used to carrying out high-risk projects on its own that experience has been disappointing. So the corporation is looking to the food industry and elsewhere in biology-based industry where indigenous research tends to be short-term.

Cain acknowledges that NRDC spending will continue to be higher in the physical sciences because such projects tend to call for the building of expensive large-scale prototypes. But the time may come when NRDC will have to build biotechnical pilot plants, which will call for larger resources. "It's quite easy to imagine a £1 million project with industry", he says, "which aimed to go commercial in three or four years."

The new interest in biotechnology does not however mean that the NRDC's traditional interests will vanish. In particular, the corporation will continue to be heavily involved in computing machinery and in computer software. But for the time being, it seems to have an edge in biological engineering.

Robert Walgate

Ariane

Getting bigger and better

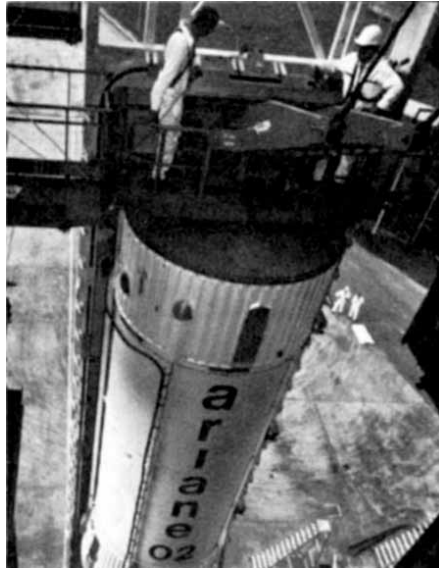
THIS is yet another milestone-week for *Ariane*, the satellite launching system developed by European governments under the aegis of the European Space Agency (ESA). Tomorrow, 23 May, the second test-flight of the *Ariane* system will be carried out from the rocket base in French Guiana. Today, 22 May, the Council of the ESA is meeting to decide how the cost of the next three years' development, estimated at 175 million units of account (about £115 million) should be shared among member states.

The plan is to improve *Ariane* to carry payloads of 2,000-2,300 kg, compared with the 1,750 kg capacity of the current launcher. The new systems will be capable of placing two 1,000 kg satellites into geostationary transfer orbit and so will fulfil most of the requirements of launching heavy telecommunications satellites.

The development is planned in two phases and *Ariane* II and III should be complete by 1983. *Ariane* II will be developed first to carry payloads of up to 1,950 kg by increasing the thrust of the first and second stage engines and the propellant mass of the third stage. *Ariane* III, with a payload of 2,300 kg, will have two boosters fitted to the first stage. The volume available for carrying satellites will be increased in *Ariane* III to allow two satellites to be launched together, reducing costs.

The stimulus for the *Ariane* programme has come from the Centre National d'Etudes Spatiales in France (France has funded 55% of the 790 million accounting units of the first phase of *Ariane* development over the past seven years). CNES has done much of the groundwork for *Ariane* II and III and is already planning later versions. According to M Bouillard, head of advanced projects at CNES, *Ariane* IV, which would be *Ariane* II with four extra boosters and a 35% increase in first stage development and would accommodate payloads of up to 3,500 kg, could be developed by 1985. Plans for *Ariane* V, however, are more ambitious. It would be capable of putting into orbit a manned vehicle which would return to earth under its own steam. CNES is also looking at the feasibility of recovering and re-using some components of *Ariane* V. It hopes to develop *Ariane* V by 1990.

In the meantime, the development of *Ariane* I is coming to an end. Earlier this year ESA handed over routine production to a consortium of European industry, Arianespace. After the four test launches (the last is scheduled for early 1981) and two subsequent launches, ESA will be free



Ariane II, second stage in the development of the ESA's satellite launcher, to be launched from French Guiana tomorrow.

of financial responsibility for further production, provided that Arianespace is financially viable. ESA member states are currently signing an undertaking to help bail Arianespace out should it ever get into trouble.

ESA will, however, retain responsibility for maintenance of the *Ariane* launch pad at Kourou, French Guiana. Member states will also decide at this week's council meeting whether to fund the building of a second pad at the site. The existing pad can handle only four launches a year. When the larger versions at the rocket come into operation, ESA estimates that up to six launches will be needed each year.

Even if, as expected, the plan for *Ariane* III is approved this week, the budget will be considerably less over the next three years than it was at the height of the development of *Ariane* I. It is unlikely, however, that much of the money released will be channelled into ESA's other programmes, in particular the science programme, which desperately needs more funds. Any increase in the science budget, which is mandatory for all member states, would have to be approved by them all. Those who do not contribute to *Ariane* are unlikely to agree to spend more.

ESA's immediate concern, though, must be the success of the second *Ariane* test-flight, scheduled for 23 May. The two small German satellites, "Firewheel" and "Oscar 9", carried free of charge, are also dependent on the success of the mission. "Firewheel" is a small satellite built by the Max Planck Gesellschaft to release barium and lithium clouds into the magnetosphere and monitor their movement through it. "Oscar 9" is a satellite for radio hams. If the flight goes as well as the first test, ESA and the German satellite operators should have few worries. Member governments may also pay up more cheerfully.

Judy Redfearn

Grant audits

No easy riders

Washington

THE dust is refusing to settle around conflicts between research universities and government accountants over the auditing of federally-supported research grants. It is more than a year since the Office of Management and Budget (OMB) issued the final version of its revised accounting rules, a document known as Circular A-21, hoping for a temporary pause in the skirmishing around grant accountability.

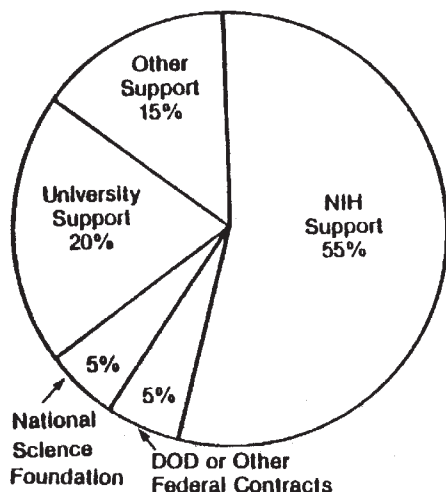
But complaints continue to mount, particularly over the requirement for detailed records of how scientists and technicians spend their time. The government's obvious interest is that funds allocated to a project should not be spent on anything else.

The suggestion is now gathering momentum that tensions may be resolved only by radical changes in the basis of research grants. One possibility is a shift from the "cost-reimbursement" basis to a fixed-price, grant-in-aid system, under which, once a grant has been awarded, research workers and their universities would decide how it should be spent.

Ample evidence of continuing frustration was voiced in Washington last week at a meeting of the advisory committee to the Director of the National Institutes of Health. It was plain that financial stringency has also exacerbated tensions between scientists and their universities.

The issue is the level of overhead costs universities should be entitled to add to research-grant proposals. Overhead percentages have increased rapidly in the past decade. The proportion of NIH grants spent on indirect costs rose from 28.5 per cent in 1970 to 41 per cent in 1979, according to a survey carried out by the National Eye Institute. On the grounds that when overall budgets are static, rising overheads mean less money for research proper, the council of the Eye Institute recommended last October that the NIH should reduce all indirect-cost allowances by a third. And the University of California's faculty Senate has rejected proposals from the University President for an increase in overhead percentages.

University administrators firmly resist such reductions, insisting that indirect costs have increased dramatically, largely, they claim, because of federal regulations, for example covering affirmative action or occupational safety. They claim that most universities are able to collect only 80 per cent or less of the costs incurred in undertaking federal research commitments. At Washington University, according to Chancellor Dr. William Danforth, the total costs of carrying out federal research and training in 1978-79 was \$49.6 million, but only \$40.4 million was recovered from the government.



NIH estimate of the sources of income of a typical pre-clinical research laboratory in the United States (percentages of total).

More immediately troublesome for research workers is the need to give precise estimates of how much time they spend on projects.

Circular A-21 relaxed the time-and-effort demand by agreeing that faculty members need only complete such a record at the end of every term rather than every month (reports on technicians are still required monthly), and offering the alternative of "workload monitoring" where time allocation is agreed before a project is begun.

But research workers still find the system oppressive. Many claim it is unreasonable, for example, for a research scientist in a medical school to decide how time has been distributed between functions that may have been carried out simultaneously. "It is impossible to allocate one's time between research, training and patient care, and it is a sham to try to impose it," Dr David Kipnis, chairman of Washington University's School of Medicine, told last week's meeting.

Others argue about the difficulty of shifting from the rigours of scientific quantification to the "creative fictions" of the accountant. "We put a high degree of rigour on the numbers that we generate. So when we get a form asking us to put down numbers, we tend to think that they are the same type of numbers, ignoring the fact that they may not correspond to our notion of reality," said committee member Dr Howard Temin, professor of oncology at the University of Wisconsin.

Government auditors are unbowed. They insist that the revised A-21 requirements have considerably reduced the reporting load. They also point out that current auditing requirements follow directly from the way Congress has chosen to support biomedical research.

This has prompted increasing suggestions that alternative ways of supporting research should be explored. In a report on funding mechanisms soon to be published, for example, the National Commission on

Research (NCR) suggests that experiments be made with locally-managed grants-in-aid, shifting the emphasis from detailed financial accountability to scientific accountability.

Under such a system, a grant proposal would still be closely scrutinised by the funding agency using the full peer review process. But once the money had been allocated, the investigators' prime responsibility to the agency would be to report on the technical aspects of the research. Administrative details would become the responsibility of the investigator and his or her university, with the funding system becoming the main incentive for the wise management of resources.

NIH is keen to encourage experiments along these lines for certain types of grants, according to director Dr Donald Fredrickson, while OMB officials say that, in principle, such a basis would be acceptable and simpler to administer.

In a different context, the National Science Board, the governing body of the National Science Foundation, has given some thought to the merits of block-grants to universities, so far without conclusion.

Not all universities relish the prospect of having to decide how a research worker is entitled to spend a federal grant. In addition to the increased administrative load, one result could be to translate current tensions between scientists and government accountants into the university setting. And there are several procedural issues, such as the way that indirect costs are calculated (currently as a proportion of the salaries and wages included in the grant) that would need to be revised.

The NCR, a body set up two years ago to examine all aspects of research funding, considers that these changes could work. But it urges the need for experiments. The National Science Foundation is already experimenting with post-award aggregation of grants to a selected number of university chemistry departments. The NIH, which supports almost 50 per cent of federally-sponsored university research, is planning to follow suit.

David Dickson

PWR design

UK programme gathers steam

DESIGN work on modifying the Westinghouse pressurised water reactor system to meet British safety standards has begun "in earnest" following a letter of intent issued by the UK Central Electricity Generating Board to the Nuclear Power Corporation late last month.

The NPC is increasing the staff of its light water reactor team from 80 to 170 by

the end of September, and plans a total of 400 by the end of 1982. Design staff, who are not nuclear experts as such, are being recruited from engineering fields associated with complicated plant design including piping engineers, quality control draftsmen, power engineers and computer specialists. In addition the NPC is transferring part of its existing staff of 1,200 to PWR work. Included in this number are engineers from the advanced gas cooled reactors located in Hartlepool, Heysham and Dungeness. Two NPC engineers will fly to Pittsburgh next month to work with a Westinghouse licensing group on the transfer of the details of PWR technology to the UK. Five Westinghouse engineers will join the NPC team next month to work on modification of the Westinghouse design to meet UK standards. Technical support from Westinghouse will grow to about 100 professionals by 1982.

NPC staff is concentrating its present efforts on computer simulations of safety procedures and on physical modelling of the layout of the 1100 MW reactor. The design of steam, water, gas and cable runs is to conform with British safety procedures developed through experience in petrochemical plant design.

In conjunction with engineers from the CEBG, the NPC will prepare its modifications of the Westinghouse system which includes a novel "double skin" consisting of two concentric concrete cylinders with the reactor hardware in the inner cylinder and the safety and standby systems in the outer cylinder to meet operator radiation dose standards and plant standby power requirements, and to "relieve burden on the human element". The design work will cost £3.5 million.

The Nuclear Installations Inspectorate says that it expects to receive the CEBG's preliminary safety report — a general outline of the standard safety procedures to ensure reactor safety — within the next few months. This will be followed by a "steady flow" of design information beginning with a safety analysis of the main design followed by fault analyses of particular areas ranging from pressure vessel integrity and the possibility of fast fracture from small cracks (*Nature*, 28 February, p804) to the reliability of used equipment and component failure records. In spite of staff shortages, the NII expects no difficulty in meeting its obligations to evaluate PWR safety.

Approval of the design modifications by the NII by 1982 will be followed by an application by the CEBG for a nuclear site license at the Magnox reactor site at Sizewell. At the same time, the promised public inquiry will also be announced and the safety documentation will be released in accordance with the government's announcement on 18 December 1979 that "principal safety documentation relevant to the initial licensing will be published and made available before the inquiry".

Jo Schwartz

Soviet psychiatry

Mock trial in London

THE second anniversary of the arrest in the Soviet Union of Alexandr Podrabinek was marked in London last week (15 May) by a "public hearing" on the alleged abuse of psychiatry in the Soviet Union. Podrabinek is the author of the *samizdat* treatise "Punitive Medicine" and a member of the organisation known by the cumbersome title of Working Commission for the Investigation of the Misuse of Psychiatry for Political Purposes.

The chief object of last week's hearing, at which Mr Louis Blom-Cooper Q.C. put the case for the prosecution, was to draw attention to the plight of the working commission, only two of whose original members remain at liberty in the Soviet Union. Other members of the commission now arrested include the mathematician Vyacheslav Bahkmin (12 February, 1980) and the physician Leonard Tarnovskii (4 April, 1980). The pharmacist Viktor

Nekipelov, who was a member of the Moscow Helsinki Monitoring Group, had worked closely with the commission until his arrest on 7 December last year.

At last week's proceedings, one of the chief witnesses was Dr Alexandr Voloshanovich, who had been forced to emigrate to Britain in February this year. He told the hearing that he had investigated a total of forty alleged victims of psychiatric abuse before his departure from the Soviet Union, but that in no case had he found "indisputable evidence of the need for hospitalization".

Interest in the West has necessarily centred on the more spectacular cases of alleged psychiatric abuse, such as the hospitalization of Petro Grigorenko for his defence of the Crimean Tatars (and whose cases was simulated in last week's mock trial). Dr Voloshanovich emphasised, however, that the cases which he had investi-

gated were varied, and that in his experience some of those sent to psychiatric hospitals had not given political offence but, rather, had simply been trouble-makers for the system.

Mr Peter Reddaway, co-author of the book "Russia's Political Hospitals", suggested that psychiatric abuse has featured "quite prominently" in recent arrests of those known to have dissident sympathies. Of 200 arrested since last autumn, he said, fifteen have ended up in psychiatric hospitals.

Even so, there are some signs that the Soviet authorities are sensitive to allegations of the misuse of psychiatry in the Soviet Union. When the Royal College of Psychiatrists (London) wrote at the beginning of the year to Dr Andrei Snezhnevskii asking for an explanation of the growing number of reports of psychiatric abuse in the Soviet Union, Snezhnevskii offered no explanation but did resign his honorary membership of the college.

Vera Rich

Antarctic ecology

Monitoring krill from land

THIS season's Polish expedition to the Antarctic returned home two weeks ago claiming that it should at some stage be possible to monitor the exploitation of Antarctic krill by observation of the land-based fauna of the Antarctic coast. This is one of the arguments that Polish ecologists will be putting to the forthcoming conference in Paris on Antarctic biomass.

The Polish Antarctic base "Henryk Arctowski" was established in 1976 on King George Island in the South Shetland Islands and can accommodate 70 people in summer, with up to twenty people over-wintering. Given the location of the station, Polish concern with the oceanic ecology of the Antarctic is understandable.

Dr Stansilaw Rakusa-Suszczewski, of the Polish Institute of Ecology and co-ordinator of the biological part of the Antarctic programme, said after the expedition had returned that Poland has no ambition to exploit Arctic krill commercially, but that for the time being at least is concerned only to understand more clearly the somewhat fragile ecology of the Antarctic.

The chief objectives of the biological part of the past season's Antarctic programme were to understand the flow of nutrients off the coast of Antarctica in such detail that marine production could be accounted for. Previous Polish expeditions to the Antarctic have drawn attention in their reports to the way in which marine production is determined jointly by off-shore upwelling and the ex-

change of material between the sea and land surfaces.

Dr Rakusa-Suszczewski now thinks that it will in due course be possible to follow the production and exploitation of oceanic krill, and of other marine animals occupying lowly positions in the food-chain — almost literally low in the oceanic pecking order — simply by maintaining regular counts of the populations of penguins and seals living on the Antarctic coastline.

Marine ecologists will no doubt await the publication of the past season's results before accepting Dr Rakusa-Suszczewski's claim. Penguins and seals are known to be in-shore feeders, and the dependence on what happens in the deep

Antarctic must be tenuous, to say the least of it.

Nevertheless, in strict compliance with the formal terms of science planning in Poland, the Antarctic programme of research is aimed at the solution of an "interdepartmental problem", defined as such because it integrates "environmental and biological research with the objective of understanding the dependence and functioning of part of the ecosystem and the inflow of material in the inshore zone of Admiralty Bay".

In case all the subtleties of Antarctic ecology can be worked out only after further painstaking research, Dr Rakusa-Suszczewski is also at pains to emphasise that the past season has produced some practical benefits — the possibility that fish-bile may be a valuable source of cholic acid for the pharmaceutical industry, for example.

Vera Rich



Henryk Arctowski — no krill in sight

CORRESPONDENCE

High book prices

SIR, — It is not just a berserk economy that is to blame for the high price of books. A colleague of mine expressed horror at the price proposed for a hard-back that he had intended for students, and asked why it could not be printed as a paperback. The answer was: "If it sells well then we shall publish a cheap edition". The Russians must be laughing at our marketing.

Yours faithfully,

PHILIP J STEWART

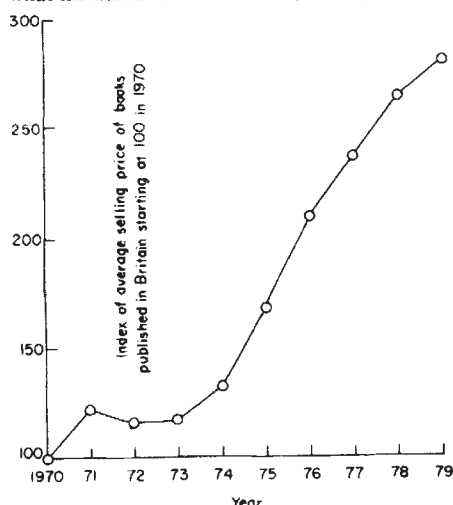
University of Oxford, UK

SIR, — The leader entitled "Are Books too Expensive" (24 April page 649) was a wayward attempt at analysing a problem of great concern to everyone involved in publishing.

We were told that for the price of a skipped lunch we could once walk out of a bookshop with a few titles under our arm; perhaps we could be supplied with the actual year when this was possible. Let us assume it was 1962, before the boom in science publishing. I asked two colleagues who had not read the article how much a cafeteria lunch of say three courses might have cost in 1962; one said 3/-, the other said 2/6. I then checked records for the prices of monographs published in that year; a book of 256 pages cost an average 42/-. To walk out of the shop with one monograph, the author would therefore have had to skip 14 3/- lunches.

How many lunches would the author have to skip now? We have just published a monograph of 256 pages at £15.00 and a simple three course lunch seems to cost around £1.25. The author would actually have to skip two fewer lunches today!

For those who would like a more serious analysis the graph (based on figures supplied by the Publishers' Association) shows how the average price of books published in Britain has risen since 1970. In the same period the UK Retail Price Index has risen a little more, just over 300%. Average earnings have increased by more than the RPI since 1970, but perhaps what the author should be complaining about



is that the average earnings of the book-buying population have probably slipped in relation to the rest of the population.

It is suggested that we should show restraint over symposia and monographs and avoid duplication. Symposia and monographs can, in fact, be published profitably if overheads are kept to a minimum as explained in the *New Scientist* (21 June 1979 page 1013) and the right number is printed, but most

publishers are leaving these to a few companies which concentrate on specialist works. The vast majority of publishers would prefer to sell more copies of fewer titles but as sales of individual titles are decreasing, the response from some publishers has been to publish more titles to maintain turnover. The Soviet Union avoids duplication by controlling publication through central committees. The members of these committees are undoubtedly exceptionally talented but as the author points out, such a system cannot always be right in predicting which book will be essential to the development of a subject.

The real problem for British-based science publishers is that, although they are trying to hold down their prices, the value of the pound abroad is hitting the export market which takes around 60-75 per cent of production. Our books are becoming too expensive in some overseas markets. Our export market has also been weakened by the rise of indigenous publishing houses moving from producing translations of successful English language texts to books written by local authors. Students are prepared to pay more for texts in their first language and aimed specially at their courses. This is a healthy development in publishing as a whole, but it means that the publishers working in the English language can no longer assume that their books are automatically international products and the first choice for students in many parts of the world.

Publishers can adapt to the problem of high selling prices resulting from the high value of the pound abroad by producing abroad; indeed many are already doing so. This however could permanently damage the UK printing industry. The current wave of redundancies in publishing could be quickly followed by further unemployment amongst printers.

Yours faithfully,

ROBERT CAMPBELL,

Blackwell Scientific Publications
Osney Mead, Oxford, UK

No gas guzzling

SIR, — In your leading article of 17 April about energy conservation, you say that the "Gas Board . . . advertises to encourage us to use more energy".

The fact is we do precisely the opposite — and just what you advocate. Your article is headed "Conserve energy, conserve cash". One of our slogans, widely used, is: "Save Gas, Save Money". Our publicity has plugged an energy conservation line for many years now. Moreover, since early last summer, no promotion of gas or gas appliances has been pursued that would build up gas load at the peak. On the contrary, television advertising about using gas efficiently has appeared throughout the winter months and is being followed by a campaign on similar lines in the national press at present. The efficient use of gas is one of our key objectives.

Yours faithfully,

F W PLOWMAN

Public Relations Department
British Gas Corporation

Responsibility for nuclear weapons

SIR, — Despite rising international tension there is little public debate about Britain's policy on nuclear and other modern weapons, especially in comparison with the impassioned campaigns for and against nuclear energy. And yet while nuclear energy is at worst a

long-term threat to the quality of our civilisation, nuclear weapons could obliterate it in half an hour.

We have lived quietly with this peril for more than twenty years, during which the policy of deterrence has not failed. But there are several disturbing new developments that require urgent discussion, especially by scientists. First, the government's decision to consider producing chemical weapons; second, the government's decision to consider spending £5,000 million replacing Polaris nuclear submarines and missiles with the Trident system; third, the government's reaffirmation that its policy is to offer virtually no protection to civilians in the event of war; fourth, the government's decision to allow cruise missiles to be stationed in Britain; fifth, the increased accuracy of guidance systems (which could tempt the Russians or Americans into considering a first strike against each other's missiles).

The scientific community must play an important part in the extensive discussions that these ominous developments ought to provoke. In particular we should put under close scrutiny the activities of our colleagues who carry out weapons development. Lord Zuckerman has argued first, that their technical innovations have created a new situation, undermining deterrence by making all-out war a seriously-considered policy option, and, second, that the implementation of new weapons systems is often at the instigation of scientists rather than of the military. No longer should it be claimed as in the Second World War that military scientists are helping their side to win, nor, as in the 1950s that they are building up an effective deterrent. Instead, it should be recognised that the contribution of scientists to warfare has entered a third phase, in which their prolific inventive capacity is itself a powerful destabilising influence.

The consequences of the arms race continuing unchecked are so dire as to make it imperative for us to overcome the paralysing hypnosis induced by our sense of helplessness as individuals. As a small beginning we invite scientists to take the following steps:

1. To oblige those of our colleagues who devise and advocate new weapons of mass destruction to stand up and justify their work (as their counterparts in the nuclear energy industry are having to do).
2. To inform our students (and ourselves!) of the far-reaching and complex relationship between military technology and progress in 'pure' science.
3. To initiate informed discussion on weapons development in our various institutions and professional bodies and to make our own view clear. Those opposed to further weapons development might consider discouraging students from applying to enter military research establishments.
4. To support currently faltering international attempts towards arms control.
5. To promote public discussion by making MPs aware of our fears and encouraging them to play an active role in forming sane weapons policies.

Yours faithfully,

MICHAEL BERRY,
ALWYN EADES,
DAVID FIELD,
TIMOTHY POSTON,
GORDON REECE,
JOHN ZIMAN,

Bristol University, UK

JOHN CHARAP,
Queen Mary College, London, UK

NEWS AND VIEWS

The Cretaceous-Tertiary boundary event

from Finn Surlyk

SIXTY-FIVE million years ago a major crisis hit the flora and fauna of the earth. In stratigraphic terms this took place at the boundary between the Cretaceous and Tertiary systems. Evidence for the crisis lies in the more or less simultaneous extinction of major groups of animals and plants including such characteristic elements of Mesozoic life as the dinosaurs, the great marine reptiles, the flying reptiles, the ammonites and belemnites, many bivalve groups, and major groups of marine phyto- and zooplankton^{1,2}. It has been estimated that as many as 75% of Cretaceous species may have been eliminated.

Searching for the cause of this event has been a favourite pastime for a large number of scientists and a great number of hypotheses have been put forward. Some observations suggest a catastrophic mechanism whilst others are indicative of more gradual processes¹⁻³.

In the last few years the catastrophists have dominated the scene. The main reason for this is the extremely abrupt wholesale extinction of major groups. This is well known from the type area of the lowermost Tertiary, Danian Stage in Denmark^{4,5}, from several sections in Spain⁶, from the Italian Apennines⁷, and from many holes of the Deep Sea Drilling Project^{8,9}.

The catastrophists of today seem to have focused on two major hypotheses. The first one is of terrestrial nature and comprises spill-over of brackish water from an isolated Arctic Ocean at the time of opening of the Greenland Sea — Norwegian Sea passage by sea-floor spreading^{8,10}. Thierstein and Berger⁸ supported this theory by carbon and oxygen isotope data, while Gartner and Keany¹⁰ used nannoplankton from North Sea chalk to support the "spill-over" hypothesis. In the latter case an isolated occurrence of a 'Danian' nannoflora was

found in a core 55 m below the supposed top of the late Cretaceous, Maastrichtian stage. This was taken to suggest that 'Danian' coccolithophores and planktonic foraminifers developed during late Cretaceous times in an isolated Arctic Ocean having a greatly reduced salinity. An initial, temporary opening of the Greenland Sea thus accounts for the lowermost occurrence of "Danian" coccoliths in the late Maastrichtian.

The North Sea section was, however, reinterpreted by Perch-Nielsen *et al.*⁵ and by Watts *et al.*¹¹ who clearly demonstrated that the Maastrichtian-Danian boundary actually occurs below the lowest 'Danian' interval and that the overlying 55 m of Maastrichtian chalk actually represents slide material redeposited in Danian time. While this work certainly invalidates the data base for the Gartner-Keany hypothesis, it does not necessarily eliminate the spill-over hypothesis.

The second major catastrophic hypothesis is of extraterrestrial nature involving the influence of a supernova, meteorite or comet. While this idea is not new it has had a considerable revival in the last few years due to the discovery by Alvarez *et al.*⁵ of anomalously high levels of iridium at the Cretaceous-Tertiary boundary in several sections near Gubbio, Italy. Ir in crustal rocks is typically 10^{-3} of solar system levels, so high Ir could indicate an influx of extraterrestrial material. Ir isotope measurements show less than a 2% difference from the terrestrial Ir ratio suggesting that the Ir came from a solar system source, not a supernova.

The same anomalous trace element enrichment, especially of iridium and osmium at the time of extinction was recorded by Smit and Hertogen^{5,6} (this issue of *Nature*) from an almost complete sequence across the Cretaceous-Tertiary boundary in Caravaca, SE Spain. This was

taken to support the idea of a large meteorite collision.

Hsü, also in this issue of *Nature*, has used the extinction and trace element data from Gubbio and Caravaca to advance an extreme theory¹² that suggests the extinction of the large terrestrial animals was caused by atmospheric heating during a cometary impact. Poisoning by cyanide released by the fallen comet and a catastrophic rise in the calcite compensation depth in the oceans are separately proposed as the main agents responsible for the extinction of the calcareous marine plankton. Several tests are possible for this hypothesis. First, where is the crater? Hsü assumes that the meteorite fell in the ocean, and since much of the Mesozoic ocean floor has disappeared by subduction, the crater may have vanished. The instantaneous extinction of the large terrestrial animals offers another test possibility. Where are all the accumulations of both vertebrate and invertebrate skeletons at the boundary? It may of course be argued that some of the terrestrial skeletons were destroyed by burning, but, if found, such fossil populations should have a characteristic age distribution indicating mass-mortality of the whole population.

A very recent paper on the Gubbio sequence by Wezel¹³ should dampen the enthusiasm of the catastrophists.

The key geological section for the catastrophists is situated at Bottaccione near Gubbio in the Italian Apennines. It is generally interpreted as an essentially complete sequence of Middle Cretaceous to Palaeocene calcareous, pelagic sediments¹⁴. Palaeomagnetic studies of the Upper Cretaceous and Tertiary parts have yielded a sequence of magnetic polarity zones, which are said to correspond

Finn Surlyk is in the Geological Museum of Copenhagen University.

precisely with the marine magnetic polarity profiles. They are dated by foraminifera⁷, and the section is proposed as a magnetostratigraphic type section¹⁵. The Bottaccione data have furthermore been used to recalibrate the existing magnetic time scale and thus to revise the oceanic spreading rates. The tectonic rotation of the Italian peninsula as an independent microplate or as part of the African plate is clearly seen in these magnetic data. Finally, trace element anomalies mentioned above have been of tremendous importance in the discussion on the late Cretaceous extinction event.

The exact nature of the sequence has thus formed the basis for the catastrophist's far reaching conclusions. Data published so far on the sedimentology of the critical strata are sparse and mainly of lithostratigraphical nature¹⁴ and can not provide the basis for a detailed interpretation.

Wezel's recent detailed study¹³ of the sedimentology of the critical sequence is thus of fundamental importance. This is particularly the case as Wezel in his introduction states that "The formation turned out not to be the product of a long and continuous 'gentle pelagic rain' and also the classical and type-biostratigraphic zones were found to contain younger reworked foraminifera" and "... unfortunately things are not as simple as thought by previous researchers".

According to the detailed and convincing observations by Wezel a lot of the presumed 'pelagic' deposits are calciturbidites resedimented from currents flowing from the east. Stratigraphic changes of magnetic properties at Gubbio seem crudely to match local variations in bed thickness. This certainly rules out the possibility of using the section as a magnetostratigraphic type section for the Upper Cretaceous and Palaeocene. Finally, sedimentologic evidence suggests general reworking of microfossils with very few indigenous forms. This taken together with the very poor fossil preservation in the sequence and the extremely low macrofaunal diversity detracts very strongly from the stratigraphic value of the once celebrated section. Wezel's paper is so detailed that the burden of proof now rests on his opponents^{7,14,15}.

One feature of the Gubbio section still has to be explained; namely the anomalously high iridium and osmium contents. Since the relevant analytical

techniques are quite cumbersome, relatively few determinations of Ir and related trace elements are to be found in the literature on sedimentary rocks. The question thus arises: What do we know of the amount of iridium present in other parts of the sedimentary record? How many analyses have been undertaken and how many is it necessary to undertake before one can safely claim than an

occurrence is anomalous?

It thus seems that we are back again where we started and the end-Cretaceous extinction event still presents one of the major examples of the 'Vogt-Holden Effect': "New data, regardless of reliable source of high quality, have scarcely ruled out any past theory, but have fueled the promulgation of newer and even more outlandish proposals". □

Kaon-Nucleon physics

from David Miller

MESON-NUCLEON interactions are at the core of the old-fashioned strong-interaction theory of elementary particles and, although we now have a new-fangled strong-interaction theory of quarks and gluons (quantum chromodynamics 'QCD' see *Nature* 279, 479; 1979) the structure of the meson-nucleon scattering amplitudes still has to be studied by the old techniques. The relationship between the two theories is not very direct, but one important source of data for those who try to build hadrons (strongly interacting particles) from quarks is the spectrum of meson-nucleon resonances. These resonances appear as poles in the complex scattering amplitudes and it has been the task of experimental physicists for over twenty years to measure scattering cross-sections and polarisations at a wide range of angles and energies in order to reconstruct the amplitudes. Since the data is always limited in both coverage and precision the old-fashioned theory developed a set of mathematical tools, particularly dispersion-relations, which impose strict constraints on the analyticity and continuity of the partial-wave scattering-amplitudes. Reports were presented by Koch (Karlsruhe, Helsinki) and Cutkosky (Carnegie-Mellon, Lawrence Berkeley Lab.) on the latest results of fits to the pion-nucleon data. The general mutual agreement of these fits was impressive. Unanswered questions remain. For instance, it is not clear whether the "minimum multiplets" postulated for the quark-structure of resonances can survive; only negative-parity {70} and positive-parity {56} representations in SU(6) have so far been firmly established but Cutkosky reported a number of awkward new candidates which are hard to fit in. Nevertheless, the major structures in the lower partial waves are clear and such basic dynamical quantities as the πNN coupling constant and the "sigma term" can be quoted with some precision (e.g. $g_{\pi NN}^2 = 13.9 \pm 0.36$ — Samaranika *J. Phys. G*, 5, 657; 1979).

The structure of the kaon-nucleon and antikaon-nucleon amplitudes is by no

means so clear. Oades (Aarhus) reviewed the partial wave analyses and a number of speakers, especially from the host group, discussed the dispersion-relations, the zeros of the scattering amplitudes and the sigma terms. A few important features seem to be settled. The $\bar{K}NA$ coupling has been determined by a number of techniques which give different weight to different parts of the data. Queen (Birmingham) reviewed these results, which mostly give values for $g_{\bar{K}NA}^2$ between 14 and 17, in good agreement with the SU(3) prediction that $g_{\bar{K}NA}^2 \approx 1.08 \times g_{\pi NN}^2$. The ispin zero triplet p-wave in kaon-nucleon has a resonance-like behaviour in most fits, but it is generally agreed that it can be explained as a 'woolly cusp' a combination of an attractive potential and the opening up of the inelastic channels. New data were presented on K^+ neutron polarisation from groups working at KEK (the new Japanese proton-synchrotron) and at NIMROD (the retired British proton-synchrotron). Preliminary fits to the NIMROD data by the Queen Mary College — Rutherford group have shown good agreement with B. R. Martin's existing fit, but the KEK data did not seem so closely compatible with previous fits. These are preliminary results from both groups and it will be interesting to see how their presentation develops after the long corridor-discussions between their spokesmen (Takasaki from KEK and Watts from Queen Mary College). But, whatever the outcome, it seems that there is no need to strain the quark-models in order to account for an exotic (5 quark) K^+ — neutron resonance.

The antikaon-nucleon resonance spectrum is still quite fluid. Different fits to the data disagree in quite low partial-waves and there is even a major puzzle, the Λ (1405), below the $\bar{K}N$ threshold. Because the mass of a K^- and a proton (1432 MeV/c²) is considerably higher than that of a pion and a lambda-hyperon (1251 MeV/c²) or a pion and a sigma-hyperon (1327 MeV/c²) there can be exothermic $K^-p \rightarrow (\Lambda\pi \text{ or } \Sigma\pi)$ transitions even from K^- interacting at rest. Experiments in which $\Sigma\pi$ states are produced in association with other final-state particles have

- 1 Hallam, A. *Nature* 281, 430; 1979.
- 2 Russell, D.A. *Ann. Rev. Earth Planet. Sci.* 163; 1979.
- 3 Russell, D.A. *Episodes* 4, 21; 1979.
- 4 Birkelund, T. & Bromley, R.G. (ed.); *Cretaceous-Tertiary boundary events I*; 1979.
- 5 Christensen, W.K. & Birkelund, T. (ed.); *Cretaceous-Tertiary boundary events II*; 1979.
- 6 Smit, J. & Hertogen, J. *Nature* 285, 198; 1980.
- 7 Premoli Silva, I., *GSA Bull.* 88, 371-374; 1977.
- 8 Thierstein, H. & Berger, W.H. *Nature* 276, 461; 1978.
- 9 Boersma, A., Shackleton, N., Hall, M. & Given, Q. *Init. Rep. DSDP* 42, 695; 1978.
- 10 Gartner, S. & Keany, J. *Geology* 6, 708; 1978.
- 11 Watts, N.L. et al. *Geology* (in press).
- 12 Hsu, J.K. *Nature* 285, 201; 1980.
- 13 Wezel, F.C. Ateneo Parmense, *Acta Nat.* 15, 243; 1979.
- 14 Arthur, M.A. & Fischer, A.G. *GSA Bull.* 88, 367; 1977.
- 15 Alvarez, W. et al. *GSA Bull.* 88, 383; 1977.

A workshop on low and intermediate energy kaon nucleon physics was held at the University of Rome, March 24-29 1980.

100 years ago

A remarkable phenomenon was observed at Kattenau, near Trakehnen (Germany), and in the surrounding district, on March 22. About half an hour before sunrise an enormous number of luminous bodies rose from the horizon and passed in a horizontal direction from east to west. Some of them seemed of the size of a walnut, others resembled the sparks flying from a chimney. They moved through space like a string of beads, and shone with a remarkably brilliant light. The belt containing them appeared about 3 metres in length and 2/3 metre in breadth.

At Paris a Society "contre l'abus du tabac" has been formed, which intends to combat the excessive indulgence in smoking which has of late become the fashion in almost the whole of Europe. The Society offers various prizes for treatises on the

human health and the dangers it is subject to from excessive use of tobacco.

The Archaeological Society of Athens has purchased about half the village which stands upon the ruins of the Temple of Eleusis. The Society intends building new dwelling-house in another part, and to begin with excavations as soon as the present inmates of the village have moved.

The astronomer, Herr Rudolf Falb, well known through his theory of earthquakes, has returned from his South American exploring tour, which extended over a period of more than two years. In his researches he was led in the direction of ethnography and linguistics, and believes that he has made interesting discoveries regarding "the original language of the human race."

from *Nature* 22; May 20, 64-66; 1880.

shown a clear peak in the $\Sigma\pi$ mass-spectrum at 1405 MeV/c². Such peaks are normally attributed to resonances, and the SU(6) theory has a place for one such resonance. But 1405 MeV/c² is so close to the physical $\bar{K}N$ region that the presence of a resonance should have an effect on $\bar{K}N$ scattering at the lowest energies. An elegant K matrix formalism has been developed for dealing with these processes. A. D. Martin (Durham) gave an up-date on his fit to the formalism, using dispersion relation constraints to eke out the sparse data on low energy K⁺p collisions. Dalitz (Oxford) agreed with Martin that there was no apparent need to invoke a quark-model resonance to explain the 1405 bump. It could be produced by non-singular meson-baryon (i.e. antikaon-nucleon or pion-hyperon) interactions, complicated in this region by cross-couplings between the channels.

There is great interest in the Λ (1405) question among physicists studying strange-particles in complex nuclei. Here the binding energy brings down the effective nucleon mass, and Fermi motion adds further complications. Dover (Brookhaven) gave a review of the work that has been done on hyperfragment formation by pion and kaon beams. Little has changed since last year's hyperfragment and K⁻ conference in Warsaw (proceedings to be published in *Nuclonika*) but he illustrated how the new generation of precision spectrometer experiments is extending nuclear structure studies into this intriguing field. Batty (Rutherford) reviewed kaonic X-ray studies. On heavy nuclei no one knows how the observed level-shifts and line-broadenings should be related to low energy kaon-nucleon processes, but his collaboration (working at NIMROD) has set a puzzle by measuring a line which may be due to the 2p to 1s transition in antikaonic-hydrogen. The line is narrow and is not shifted significantly from the position predicted for it with a

Coulomb potential alone. This suggests a very weak antikaon nucleon coupling at threshold (1432 MeV/c²), contrary to the results of all the fits, including A. D. Martin's. Kumar (Mc Master) claimed that he could fit the Λ (1405) as a resonance and match the weak coupling required by the kaonic-hydrogen result, but it was not clear if his model could be made consistent with all of the available low energy KN data. If the properties of the kaonic hydrogen X-ray line are confirmed by new experiments to be done at CERN, then those of us working on low energy antikaon-nucleon collisions will have to devise totally new techniques to study the scattering cross-sections at lower energies than can be reached at the moment. We had thought that our latest results (Durham, Brussels, Warsaw, UCL collaboration) were removing the remaining anomalies and agreeing well with A. D. Martin's fit, but there are still many poorly measured channels, particularly K⁺p $\rightarrow$ $\Sigma^+\pi^0$ in which the Λ (1405) should be important, and the K matrix parametrisation remains under-constrained. $\square$

Oil geology in China

from A. Hallam

As befits a country with an impressive tally of early discoveries, there is a record of 'fire in the swamp' (evidently marsh gas or methane) in the ancient Chinese Book of Changes, written in the 11th Century BC. In the early 12th century a gas well over a kilometre deep was drilled in Sichuan Province, but the beginning of the Chinese petroleum industry dates from 1907, when a foreign crew employed by the government of the Qing Dynasty discovered Yanchang oilfield in Shanxi Province.

For most of the first half of this century oil production remained at a very modest level but since the founding of the People's Republic there has been a considerable expansion of both exploration, with the

discovery of over 160 oilfields, and production, which last year reached 106 million tons, putting China among the top dozen oil producing countries in the world. The capital city of Beijing (Peking) was thus an appropriate place for an international conference on petroleum geology.*

A measure of the importance attached to the conference by the Chinese is that the participants were granted an audience in the Great Hall of the People by Vice Premier Kang Shi En, who exhibited a considerable knowledge of the petroleum industry. He looked forward to a huge increase in production to help promote the so-called four modernisations (Agriculture, Industry, Science and Technology, and National Defence).

While the conference lectures ranged far and wide the greatest interest of the overseas participants was obviously in the Chinese contributions, because Chinese oil geology has been hitherto largely a closed book to the outside world. Three matters of especial interest may be singled out. Firstly, virtually all the oil and gas has been generated in source rocks of continental origin, and the Chinese were manifestly proud at having confounded those pundits of an earlier era who had consistently argued that significant quantities of hydrocarbons could only be generated in marine deposits. Thus the giant Daqing Oilfield in the Songliao Basin of Manchuria, which accounts for half the current production of the whole country, occurs in a region which developed as a faulted depression in Jurassic times, long after the Palaeozoic seas had retreated. By the mid Cretaceous it became established as an open lake basin, persisting into the Tertiary, in which a great thickness of organically-rich clays was deposited. Wedges of fluvial and deltaic sands in the clays, combined with anticlinal structures, provide an excellent combination of reservoir, source and cap rocks with structural traps.

A second point of major geological interest is the occurrence under the North China Plain of so-called 'buried hill' oilfields. Huge thicknesses of shallow-water carbonates of Proterozoic to Ordovician age were subjected to extensive karstification during a lengthy period of emergence from the late Palaeozoic to the early Mesozoic, with the development of numerous small and large cavities. Subsidence under tension of a zone extending SSW from Manchuria commenced in the late Mesozoic and continued into the Tertiary. The old karst landscape was deeply buried under thick Tertiary lacustrine clays and sands. The cavities in the ancient carbonates have proved to be excellent oil reservoirs, the oil having migrated laterally from Palaeogene clay source beds, as proved by the presence in the oil of spores and pollen of that age.

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Thirdly, the Chinese exhibited a substantial knowledge of the scientific literature and technology of the West, all the more impressive bearing in mind the setbacks of the Cultural Revolution. In fields as diverse as sedimentological facies modelling and organic geochemistry they demonstrated that they had kept well abreast of modern developments. Their geophysicists, furthermore, are well ahead of the Russians in having adopted modern digital methods of seismic data processing.

Perhaps the sphere in which we in the West can offer the most help is in advice on organisation. At present the Chinese geologists and geophysicists operate independently. Efficiency could be vastly improved by integrated team efforts, for instance in the new and rapidly advancing field of seismic stratigraphy. Furthermore, it is rather strange that, while the Oil and Gas Corporation is responsible for the great bulk of the exploration and production efforts, the Ministry of Geology (effectively the Geological Survey) operates quite independently its own exploration programme both onshore and offshore.

One of the highlights of the meeting was a visit to the Renqiu Oilfield 160 km south of Beijing, which produces some 10 million tons per year from buried-hill reservoirs. This was the first-ever visit by foreigners and was accompanied not only by Chinese television camera crews and representatives of the Peoples Daily but an odd-looking assemblage of foreign pressmen, who seemed more interested in the sociology than the science or technology. The American and European

*The conference was sponsored by the China National Oil and Gas exploration and Development Corporation and the United Nations Department of Technical Co-operation for Development.

oil geologists were amazed to learn that this oilfield is quite typical in possessing its own research laboratory, equipped with the most modern petrological and geochemical apparatus. Even the microfossils are identified, as it were, on the spot. Some scepticism was expressed about how even a major oilfield could keep the laboratory workers usefully employed for long periods in more than routine monitoring.

Intensive exploration for hydrocarbons has so far been largely restricted to the eastern part of the country, although large gas discoveries in Sichuan have made the province self sufficient in that commodity. While there are some promising prospects in the vast region of Xinjiang, notably in the Tarim Basin, the greatest hopes in the immediate future are in offshore areas. The Chinese have for some time been operating drilling rigs in the Gulf of Bohai, and extracting oil from deeply buried, tilted fault blocks created by tensional collapse in the Tertiary and reminiscent of the major oil-producing structures in the North Sea. Within the last year they have invited a number of major North American and West European companies to shoot seismic lines in the Yellow and South China Seas. The results will be analysed by Chinese specialists, who will make recommendations to the Ministry of Petroleum Industry. Representatives of the Ministry will shortly be meeting with their opposite numbers in Norway and the United Kingdom, presumably to obtain advice from those experienced in granting offshore concessions. Thus we may look forward to the possibility of companies like Exxon and B.P. drilling in Chinese water, a measure of the modern pragmatism that should cause the Gang of Four a further turn in their metaphorical grave. □

sequences of integral membrane proteins with their largely hydrophobic composition has required the development of special procedures, different from those currently used for water-soluble proteins. The complete sequence of the 247 amino acids of bacteriorhodopsin was found by Ovchinnikov and colleagues (*FEBS Letters* **100**, 219; 1979) and large portions have been confirmed by Gerber *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **76**, 227; 1979) and Walker *et al.* (*Nature* **287**; 1979). As expected the polypeptide chain has long tracts of hydrophobic residues that represent the transmembrane helices; these were used by Ovchinnikov in a preliminary attempt to fit sequence to structure. However the methods used by Henderson and his colleagues are somewhat more sophisticated and warrant detailed consideration.

An important preliminary to combining the sequence and the structure, was to find the molecular boundaries for a single bacteriorhodopsin molecule. This could be done because detergent treatment of the purple membrane produces *in vitro* a new two dimensional crystal form of bacteriorhodopsin. Electron diffraction analysis of this form in projection (Michel, Oesterhelt & Henderson *Proc. natn. Acad. Sci. U.S.A.* **77**, 338; 1980) shows an identical protein structure to that observed in the purple membrane, confirming the original molecular boundary.

The first step was then to locate the seven helical segments in the sequence. This was done by making use of the known points of proteolytic cleavage which must occur at surface residues, and of the similar lengths of the seven transmembrane helices, which on energetic grounds must be largely hydrophobic. In the assignment proposed by Henderson there are 27 charged residues on the membrane surfaces and 9 buried in the interior of the membrane. The neutralization of charge by pairing these 9 buried residues becomes a major determinant in the structure prediction.

The second step was to attempt to identify the seven helical segments in the sequence (labelled A to G) with the seven helical segments in the image (labelled 1 to 7). Because the orientation of the protein in relation to the inner and outer membrane surfaces had already been established, there are 7!, or 5040, ways of combining the predicted and observed helices. To reduce this number to more manageable proportions, Henderson and his colleagues filtered the models sequentially through a number of criteria, a procedure broadly similar to that currently used in predicting the tertiary structures of proteins. The first filter was concerned with minimizing the lengths of the six interhelical loops, and disallowing loops that crossed over one

Predicting a membrane protein

from Colin Blake

ONE of the most elegant and original structural studies of a biological system in the recent past has been that of Henderson and Unwin (*Nature*, **257**, 28; 1975) on the 'Purple Membrane' of *Halobacterium halobium*. As the protein component of the membrane, bacteriorhodopsin, is a single molecular species organized into a two-dimensional crystalline array they were able to use electron diffraction to produce a three-dimensional low resolution image of the membrane protein *in situ*. Bacteriorhodopsin was revealed as an integral membrane protein composed of a cluster of seven almost parallel helices oriented perpendicular to the membrane surface.

This low resolution image was fascinating but to understand the general principles of membrane proteins and the

function of the purple membrane as a light-driven proton pump a model with atomic detail was required. This could be achieved either by increasing the resolution in the electron diffraction pattern, or by using the existing image as a basis for predicting the structure. The latter approach has been successfully used by Engelman, Henderson, McLachlan and Wallace (*Proc. natn. Acad. Sci. U.S.A.* **77**, 2023; 1980). Although less direct than extending the resolution of the image, prediction of the bacteriorhodopsin structure brings together a number of principles of protein and membrane structure and perhaps represents the more fruitful direction for future work on membrane systems.

Knowledge of the amino-acid sequence of bacteriorhodopsin was essential to either approach. Determination of the

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another on the same side of the membrane. This reduced the number of models to 405 which were rank ordered according to the total length of the interhelical loops. These models were then subjected to the test that the nine buried charged residues should geometrically be able to form ionpairs. This reduced the number of models to 405 which were rank ordered according to the calculated electron densities of the seven predicted helices with the integrated scattering densities of the helices observed in the image of the purple membrane protein. In the image helices 1 and 5 have significantly lower scattering densities than the other five. Of the predicted helices, helix D with five glycines and no large hydrophobic residues has the lowest calculated electron density, while helix A also has much lower density than the other five. This test revealed that the model with the shortest interhelical loops and the

geometry required to neutralize the charges of the buried residues, was also one of the few to meet the helix scattering criterion.

Not only does this one model of bacteriorhodopsin emerge as by far the most probable, it also possesses a number of features that seem suitable for a proton pump. The buried charged residues form one or more networks of ion-pairs through which protons may be able to jump. The retinal group probably lies near the surface of the membrane with its ring possibly buried near an aspartic acid residue. Finally adjacent helical segments in the sequence are also adjacent in the structure suggesting a particular simple folding pathway.

Confirmation of this model will be necessary, but at present it must be regarded as easily the best view we have of the structure-function relationship in a membrane protein. □

Dual polarization radar

from Thomas A. Seliga

EVER since the development of radar in the late 1930's and early 1940's, meteorologists and hydrologists have waited for the time when radar's full capabilities could be exploited for research and field operations. Progress from remote storm detection to assessment of storm characteristics has continued to the point where we can now estimate the drop size distributions of rainfall in a scattering volume (and, therefore, water content or rainfall rate), the nature of the hydrometeors producing the radar signal (whether ice particles or rain) and the bulk motion of the hydrometeors. In this issue of *Nature* Hall *et al.*¹ clearly demonstrate the first two kinds of measurement through use of the differential reflectivity (Z_{DR}) linear polarization scheme first proposed by Seliga and Bringi^{2,3} for rainfall measurements. Pulsed Doppler radar techniques have also developed rapidly over the last fifteen years,^{4,5,6} producing dramatic advances in our understanding of storm dynamics. This technique has been recently reviewed⁶, so here the impact that polarization radar measurements like those of Hall *et al.* might have in a number of areas of meteorology and hydrology will be examined. It should be noted that Canadian scientists have also developed the use of circular polarization techniques and that their results too, are of great potential importance^{7,8,9}.

Hydrology

The accuracy of the Z_{DR} technique for measuring rainfall is expected to be of the order of 30% for a single observation at a

single range gate. Since this measurement can be made within around a 200 ms time interval, and corresponds typically to an areal coverage of less than 0.5 km² for modest size radar antennas at S-band wavelengths, large areal surveillance (of the order of 35,000 km²) with high spatial and temporal resolutions of rainfall will become possible.

The resultant benefits to hydrology are many: flash flood forecasting should become more reliable; study of the evolution of rainfall in different meteorological regimes and storm types will be possible, leading to improved description and classification of storms; understanding of topographical influences on rainfall should improve; watershed and stream flow modeling should progress; rainfall in semi-arid and arid lands should be more readily monitored; and, in future, hydrologic structures should be improved as a result of more accurate, detailed information on rainfall.

Cloud Physics

Possibly the most important application of Z_{DR} in meteorology will be in studies of the organization and structure of clouds and precipitation on the mesoscale and microscale.^{10,11,12} The ability to measure rainfall accurately from a single radar site independent of rain gauge calibration, to follow the evolution of rainfall and its drop size distribution in space and time, and to accumulate and display data in real time, should provide new insights into our understanding of precipitation processes. Quantitative data of this type, when combined with Doppler data, will test the credibility of numerical cloud models and further their development. The second very important result of Hall *et al.*'s measurements is the ability to discriminate

clearly between ice and water phase hydrometeors; this will lead to improved understanding of cloud development and precipitation formation. Important data on melting, drop breakup, and the development of raindrop size distributions, should also result from such measurements.

Climatology

The development of the Z_{DR} radar technique, which can provide accurate rainfall measurements over large areas, is very important for climatological studies, particularly within the context of the World Meteorological Organizations's World Weather Watch (WWW) and Global Atmospheric Research Programme (GARP) and their subprograms.^{13,14} For example, the recently conducted GARP Atlantic Tropical Experiment (GATE) and Monsoon Experiment (MONEX) would have benefited greatly had this radar capability been available and employed. Also, use of radar for ground truth measurements of rainfall intensity and cloud structure, particularly over the oceans, should aid in the development of reliable satellite methods of estimating rainfall over remote regions of the Earth's surface. Such data is indispensable for global weather studies and validation of general circulation and climate models. Moving to smaller scales, it is apparent that regional rainfall statistics should be largely improved as radars having polarization capability become operational.

Weather Modification

Whether human intervention can influence atmospheric conditions to enhance precipitation by significant amounts remains the foremost question in weather modification.¹⁵ The importance of this problem is reflected by the Seventh World Meteorological Congress (1975) recent approval of a WMO Weather Modification Programme and Precipitation Enhancement Project (PEP).¹⁶ Again, the importance of using the dual polarization radar technique in such programs becomes evident. Accurate rainfall measurements with high spatial (1 km²) and temporal (tens of seconds)

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resolutions over large areas (35,000 km²) and hydrometeor phase detection, are essential ingredients to any such effort. Hall *et al.*'s RH1 grey scale plots of Z_{DR} and reflectivity and drop size distribution measurements show how the Z_{DR} radar might contribute to weather modification experiments. Indeed, one can contemplate using such radar data in case studies of seeded storms as a determinant for testing weather modification techniques. Whether this approach can supplant statistical methods of evaluation deserves serious attention. Another exciting role for dual polarization measurements in weather modification might arise from using Z_{DR} signatures of tracers released with seeding agents. Possible tracers include specially designed chaff and surfactants which significantly affect raindrop shape.

Other Applications

As noted by Hall *et al.*, the polarization radar should also have important applications in communications and erosion studies. Understanding the variability and characteristics of hydrometeors along terrestrial and satellite radio propagation

paths is required for systems design while erosion, both of airborne vehicles and land surfaces, depends strongly on drop size distribution. Other possible applications might include studies of precipitation scavenging of airborne particles and pollutants, hail detection, detection and classification of severe storms, and aviation radar for detecting supercooled water droplets, a precursor to possible aeroplane icing conditions.

As more radars, modified to perform dual polarization Z_{DR} measurements, become available, many of the above applications will be critically tested. The relative simplicity of the method and recent advances in high-power, microwave switching technology should enable many existing radar facilities to be modified at modest cost. This will offer many scientists opportunities to explore these ideas during this decade. The first measurements have produced dramatic results. Fulfilling all the hoped for applications is perhaps asking too much; being able to perform reliable, continuous and accurate measurement of rainfall would be enough! □

its effector in Ca²⁺ mobilising systems are not the protein components of the cell membrane but the phospholipids. M. J. Berridge (University of Cambridge) supported the idea that changes in a minor anionic species of phospholipid, phosphatidylinositol, might have this intermediate role. Several years ago it was pointed out that an enormous range of Ca²⁺ mobilising hormones also stimulate breakdown of phosphatidylinositol by a phospholipase C (Michell *Biochim. Biophys. Acta* **415**, 81; 1975). Berridge's experiments on fluid secretion by blowfly salivary glands demonstrate a correlation between the movement of Ca²⁺ ions into cells and the breakdown of phosphatidylinositol. However, some recent evidence in other tissues suggests that phosphatidylinositol breakdown may not have this intermediary role in Ca²⁺ mobilisation (e.g. Cockcroft *et al. FEBS Lett.* **110**, 115; 1980) although this may not preclude its having a different function — for example in the termination of the response.

A quite different phospholipid change was reported by J. Axelrod (National Institute of Health, Bethesda) in some of the cell types which are also known to show a phosphatidylinositol response. In these cells phosphatidylethanolamine can be converted by successive methylation to phosphatidylcholine, and this is degraded by a phospholipase A₂ to release fatty acids. In the systems examined a rapid response to stimulation has been seen. Unfortunately only a limited number of cell types have so far been studied, and it is likely that all of these cells synthesize prostaglandins from arachidonic acid as one consequence of cell stimulation. The observed phospholipid changes may thus be a means to generate the necessary fatty acid precursor from arachidonate-rich phosphatidylethanolamine; an effect specific to the cell types examined rather than a general process during receptor function.

There is yet another possibility. W. Trautwein (University of Saarland, Germany) described measurements of conductance changes associated with a muscarinic receptor in heart. The results showed considerable similarity to comparable experiments with the much more rapidly responding nicotinic cholinergic receptor (D. Colquhoun, University College, London). It is generally assumed that the nicotinic receptor molecule is itself an ion channel (in this case for Na⁺), although a completely convincing demonstration of that *in vitro* has still not been achieved. A possible implication is that for Ca²⁺ mobilising systems such as the muscarinic receptor there is no biochemical intermediate at all.

As yet the molecular understanding of

Drug receptors and their effectors

from Jonathan Bennett

How does hormonal information enter the cell? Are there intermediate biochemical events between binding of hormone to a cell surface receptor and the change in the cytoplasmic concentration of a second messenger? This was one of the problems pinpointed by the organising chairman (N.J.M. Birdsall, National Institute for Medical Research, London) at the recent Biological Council Symposium* on "Drug receptors and their effectors".

The best understood receptors in this respect are those whose cellular effects are mediated by the second messenger molecule, cyclic AMP. A few years ago evidence from various approaches forced the conclusion that the hormone receptor and its effector (the cyclic AMP-synthesising enzyme, adenylate cyclase) were separate molecular entities. It is now clear that a third protein component, a GTP binding protein, is involved (for a recent review, see Rodbell *Nature* **284**, 17; 1980). M. Schramm (Hebrew University of Jerusalem, Israel) described his experiments using a cell fusion technique to demonstrate that the GTP-binding protein acts as an intermediate between the hormone receptor and its effector. Binding of a hormone to the receptor causes the GTP-binding protein to change to an "active" form which in turn stimulates the

enzymatic activity of adenylate cyclase.

One consequence of this intermediate step between receptor and effector in the adenylate cyclase system is that it may allow other hormones and drugs to interact to modulate adenylate cyclase activity. A. J. Blume (Roche Institute, Nutley, US) described how binding of opiates to mouse neuroblastoma cells results in an inhibition of adenylate cyclase. The binding of opiate agonists to these cell membranes is enhanced by GTP, and it is a reasonable hypothesis that the opiate receptor interacts with the adenylate cyclase system at the level of the GTP binding protein.

There may be a similar interaction at the muscarinic cholinergic receptor (E. C. Hulme, National Institute for Medical Research, London) where GTP also interacts with agonist binding to isolated membranes. The effector molecule for this receptor is presumed to be a plasma membrane channel which allows Ca²⁺ ions to enter the cell — and certainly muscarinic responses belong to the large class of hormone effects which are mediated by an increase in intracellular Ca²⁺ as a second messenger. Does the interaction of GTP with muscarinic agonist binding imply that another GTP-binding protein acts as an intermediate in Ca²⁺ mobilising systems? This is a new possibility in a field where the molecular events following receptor activation have eluded critical analysis.

Alternative candidates for the intermediate step between the receptor and

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*Held at The Middlesex Hospital Medical School, London on March 31 and April 1, 1980. The proceedings of the symposium will be published as a book by The Macmillan Press.

the activation of cells by hormones which use intracellular Ca^{2+} as their second messenger lags far behind our knowledge of the adenylate cyclase system. The principal reason for this is technical — no one can yet measure changes in membrane permeability in a test tube in such a way that adenylate cyclase activities can be measured. Only when such an analysis is possible can biochemical techniques be applied. □

Platelet-activating factor

from Noel J. Cusack

WHEN IgE-sensitized rabbit leucocytes are challenged *in vitro* with antigen they secrete an extraordinarily potent, soluble, low molecular weight platelet-activating factor (PAF), which causes platelets to change shape, aggregate and release their granule contents. PAF is also released *in vivo* into rabbit plasma during the development of IgE-induced systemic anaphylactic shock and appears to be an important mediator of inflammation and allergic responses. Platelet activation by PAF is independent of arachidonic acid metabolites or released ADP. Endogenous PAF from platelets may account for that portion of thrombin and calcium ionophore induced platelet aggregation which is not prevented by blocking the ADP and arachidonic acid mechanisms, but is diminished by phospholipase A_2 inhibitors. Recent work by two independent groups (Demopoulos, Pinckard & Hanahan *J. Biol. Chem.* **254**, 9355; 1979; Benveniste *et al. C. R. Acad. Sci.* **289**, 1037; 1979) has elucidated the structure of PAF, largely by examining the effects of enzymic and simple chemical treatments on its chromatographic behaviours and its pharmacological actions on rabbit platelets.

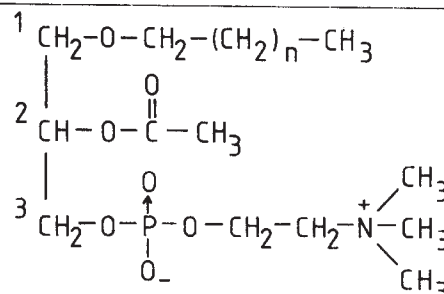
As PAF was completely extracted into organic solvents, being found entirely in the chloroform phase of chloroform-methanol-water mixtures, it was known to be a lipid. However, its insolubility in chloroform alone, coupled with a low migration on silica gel thin layer chromatography (unaltered by acidification or basification of the chromatography solvent) pointed to a neutral, very polar lipid. Possible candidates at this stage were therefore glycosylacylglycerides, neutral phosphoglycerides and neutral sphingolipids, but the lipids with known hormone-like actions (steroids and prostaglandins) could be excluded. Glycosylacylglycerides and glycosphingolipids were eliminated because PAF was unaffected by sodium nitrite, which cleaves glycosidic linkages, and by reagents for free or vicinal hydroxyl and amino groups. PAF was unlikely to be polyunsaturated because of its stability to

prolonged storage and heating in air, or to contain an α,β unsaturated ether linkage such as occurs in plasmalogens because of its relative stability to strong acid. Rapid inactivation by strong methanolic base to a chloroform-soluble derivative was evidence for at least one ester linkage, and at least one base-stable fatty group. This, together with the insensitivity of PAF to sphingomyelinase, focused attention away from sphingomyelins and onto a phosphoglyceride derivative.

Inactivation of PAF by the specific phosphoglyceride phospholipases A_2 , C and D established the presence of a 2-ester linkage and a 3-phosphate esterified to a polar head group which, since PAF was unaffected by methylating agents, was choline rather than ethanolamine. PAF was not inactivated by phospholipase A_1 (specific for 1-ester linkages) which was initially interpreted as suggesting the presence of an underivatized 1-hydroxyl group, but attempts to generate PAF-like activity by treatment of a variety of phosphoglycerides with phospholipase A_1 were unsuccessful (Benveniste *et al. Nature* **269**, 170; 1977).

Hanahan's group, exploiting the possibility that removal of the esterified group from PAF by methanolic base might provide an intermediate which could be chemically reesterified with various fatty acids to regenerate PAF activity, found that the resulting long chain fatty acid derivatives were inactive. They therefore tried esterification with short chain acids, and discovered that acetylation resulted in complete recovery of PAF activity with identical chromatographic behaviour. A 1-fatty ether structure for PAF was considered, which would remain chloroform-soluble after deesterification because of the stability of the ether linkage to methanolic base. Hydrogenation of the 1- α , β -unsaturated ether linkage of plasmalogens extracted from beef heart followed by deesterification and acetylation at the 2-position generated a compound identical in biological activity and chromatographic behaviour to PAF. Its structure was confirmed by IR and GLC as 1-O-alkyl-2-acetyl-sn-glycerol-3-phosphorylcholine with the length of the alkyl chain mainly C_{16} and C_{18} .

Benveniste's group realised that their results with phospholipase A_1 would be equally consistent with an ether linkage at the 1-position of a glycerol phosphatidylcholine, and noticed that acetic anhydride treatment increased the PAF-like activity of crude but not purified PAF preparations. This alerted them to the possible presence of an acetyl group in the molecule. Methylation, hydrogenation and acetylation of a commercial ethanolamine plasmalogen underivatized at the 2-position yielded a compound identical to PAF, and they proposed the



The chemical structure of platelet-activating factor.

same structure as Hanahan's group.

Synthetic PAF appears to be the most potent activator of platelets yet described, causing shape change, aggregation and the release reaction at concentrations between 10^{-11} M and 10^{-10} M. Interest in this novel compound must be further heightened by the discovery that antihypertensive polar renomedullary lipid (APRL), a lipid found in the kidney which lowers the blood pressure of artificially hypertensive animals, has an identical type of structure (Blank *et al. Biochem. Biophys. Res. Comm.* **90**, 1194; 1979). Synthetic APRL prepared from choline plasmalogens was an extremely potent antihypertensive agent that mimicked both the acute and the prolonged antihypertensive effects of APRL extracted from the kidney. The ubiquitous occurrence of phospholipids, together with increasing awareness of their functional importance, suggests that there may be other, hitherto unsuspected roles for PAF-like compounds. □

Life in the Precambrian

from Trevor D. Ford

THIRTY years ago the known Precambrian fossils could be counted on one's fingers. Today the number has increased to the point where a computer is needed to catalogue their names and literature references, with print-out an inch thick! Moreover they are now being used stratigraphically like Phanerozoic fossils, helping to map subdivisions of Precambrian rocks previously thought to be unfossiliferous. More importantly, they provide a record, albeit patchy, of the early history of life on earth. This was the theme of a recent symposium*.

Fossils in rocks of Precambrian age fall broadly into four categories: microscopic unicellular algae (nannofossils); clusters or associations of algal cells; stromatolitic structures in limestones (generally believed to have been constructed by algae); and metazoans (of coelenterate, arthropod or 'worm' affinities). There is great controversy about their assignment to one

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*The Symposium on 'Life in the Precambrian' was held by the Palaeontological Association in Leicester from April 10-12th 1980.

or other of the traditional Phyla, about whether or not some are fossils at all, and about their chronological relationships.

Organic-walled nannofossils extracted from sediments are generally referred to as 'acritarchs' in view of the difficulty of assigning them to any known algal groups. With some hundred or so species distinguished by their shape, size, surface ornament, wall-thickness and presence or absence of openings they have provided G. Vidal (University of Lund, Sweden) with a tool for correlation of the late Precambrian in Sweden, Norway, USSR, and Greenland. Similar studies in parts of Canada, USA, and Western Europe have extended correlation sufficiently to give a reasonably good classification of these rocks into the later Riphean and Vendian stages of Precambrian time.

The classification of acritarchs has so far lent heavily on comparisons with present day algal cells, but when various states of decay or desiccation are examined it seems that some Precambrian 'species' are, in fact, the same organism in different states of preservation. C.J. Peat (Imperial College, London) proposed that we should scrap assignments to various groups of algae and classify the Precambrian fossils into purely morphological groups of Cryptarchs. C.J. Peat, W.L. Diver (Imperial College, London) and Z. Zhongying (Nanjing University, China) also suggested that acritarch studies provide no evidence for the presence of eukaryote cells in the Precambrian; the various 'black dots' can be explained as the shrivelled inner walls of cells, or as desiccated cell contents. This argument was accepted but there must have been eukaryote algae around to allow the evolution of sex and thus of the Metazoa. When eukaryotes appeared on Earth is still a mystery and hypotheses about Precambrian atmospheric evolution cannot yet look for support from acritarchs!

That stromatolitic structures in Precambrian limestones can provide a coarse means of correlation of Proterozoic strata has been generally accepted for some years. However, being limited to shallow shelf seas, and even then with only the digitate columnar forms being useful for stratigraphy, their value is not as great as could be desired.

As shells and skeletons had not yet evolved, fossils of Metazoan life forms in the Precambrian are almost all trace-fossils or impressions; that is, they are the marks in or on sedimentary rocks made by the activities or presence of the organisms. Inevitably their interpretation can only be made by comparison with the marks made by living organisms to-day. The Precambrian Metazoan fossils include tracks, trails and burrows on the one hand and impressions of single organisms on the other. Sequences of strata below those con-

taining well-defined Cambrian trilobite body fossils have been found in various parts of the world with a host of trails, tracks and burrows. The order in which these first appear has given rise to speculation as to whether they could be used as stratigraphic markers, and whether they were representative of a fundamental evolutionary sequence. T.P. Crimes (University of Liverpool) and M. Brasier (University of Hull), A. Peréjon and M.A. de San José (University of Madrid) described the sequences of such fossils, mainly in Spain, and concluded that there was as yet no clear evolutionary sequence, but the trace type was closely related to the immediate sedimentary environment. Exact dating by radiometric methods is not yet available but it seems that no track-making animals were present in the seas before Vendian times (i.e. pre 670Ma ago).

The kind of animal that made the tracks and burrows remains a subject of speculation, but W. Gutmann (Senckenberg Institute, Frankfurt) demonstrated that penetration of sediment could only be done by animals with metameric fluid-filled hydrostatic skeletons (coelomates). Similar hydromechanical arguments supported the identification of other impression fossils as Cnidarians, some clearly of medusoid construction.

A variety of circular or oval impressions on bedding planes are now known from many parts of the world. Recent discoveries in Charnwood Forest, Leicestershire (T.D. Ford & H.E. Boynton, Leicester University), and in Carmarthenshire (J.C. Cope, University College of Wales, Swansea) include discs with few or many annular rings; are these really fossils or are they pseudofossils of the type caused by gas or water evasion from sediments? Though most agree that they are impressions made by 'medusoids' coming to rest, R.J.F. Jenkins (University of Adelaide) described scour pits remarkably like some 'medusoid' impressions on bedding surfaces but clearly penetrating the sediments when seen in section.

The abundant and varied fauna of impressions in the Conception Group of southeast Newfoundland is still undescribed in detail though found more than 10 years ago; Conway-Morris (Open University, U.K.) showed photographs indicating the presence of several frond-like 'pennatulid' *Charnia*-type coelenterates with disc-like holdfasts, as well as lobate and spindle-shaped organisms of unknown affinity. The strong lobate impressions could well represent sediment-filled gut-cavities of jellyfish, while the spindle-shaped impressions with their chevron-marked fringes could be compared with the Portuguese Man of War coelenterate.

The assemblage of medusoid, frond-like and colonial impressions has come to be known as the Ediacara fauna from the locality in South Australia where the fossils are abundant. These also include a few trails, a segmented animal (*Spriggina*) with

a crescentic head-shield, which could be either annelid or arthropod and *Dickinsonia*, probably a flat-worm. No such fossils are yet known in Newfoundland, and only one incomplete arthropod has been found in Charnwood Forest. Neither Newfoundland nor Charnwood has yielded convincing tracks, trails or burrows, so the strata there may be slightly older, signifying sequential appearance of coelenterates, followed by segmented annelid/arthropods living freely on the sediment surface, followed by those with the hydromechanics needed for burrowing. All this seems to have taken place in Vendian (or possibly very late Riphean) times, broadly within the time span of 700 to 600 Ma ago, followed of course by the explosive evolution of shelled animals in Cambrian times.

Two other long-standing problems of Precambrian palaeontology were aired: one was the true identification of the earliest known organisms in the 3 billion year old Fig Tree Formation of South Africa, where it was argued that the size distribution of the fossils did not fit normal biological patterns and that there must therefore be some doubt as to whether they were biogenic structures. The other problem was that of the *Erniettomorpha*, the host of Namibian phylloid, foliate and other petrifications imaginatively reconstructed by Hans Pflug as the common ancestors of the Plant and Animal Kingdoms. R.J.F. Jenkins (University of Adelaide) claimed them to be a single species of frond-like type in varying states of preservation; thus *Ernietta* may be no more than another pennatulid coelenterate.

The results of the symposium thus revise our knowledge of life in Precambrian times. The earliest known Fig Tree fossils are not yet proven to be biogenic; most of the acritarchs, ranging through the Precambrian, cannot yet be assigned to appropriate algal categories with confidence; the claims of eukaryote cellular nuclei as far back as 1400Ma are not substantiated; interpretation of the various medusoid impressions must proceed with caution; the sequential appearance of trace fossil types is more a matter of sedimentary facies than of evolutionary development; and the Namibian *Erniettomorpha* require much closer scrutiny.

On the positive side, it has emerged that there are plenty of Precambrian fossils if only they are looked for with appropriate techniques. New forms are being discovered in many parts of the world and stratigraphic correlation schemes are already workable, even if accurate radiometric dating is not fully supportive as yet. Every find needs close scrutiny before one can be sure that it is a fossil, and even then the placement of the organism within a biological category may raise many problems. Life in the Precambrian may be obscure, but it is not dull!

ARTICLES

Rain drop sizes and rainfall rate measured by dual-polarization radar

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A rapidly switched dual-polarization radar technique is used for the first time to obtain two-dimensional spatial distributions of the statistical characteristics of the sizes and concentration of rain drops in rain. These have been obtained using a high-resolution 10-cm wavelength radar, and the data have been used to estimate rainfall rates within small volumes to a much greater accuracy than is available from conventional radar measurements. The technique also gives a clear distinction between ice particles and rain drops.

THIS article shows some of the potential of a new radar technique that characterizes rather than assumes the distribution of rain drop sizes within a given volume, to estimate rainfall rates with improved accuracy. Many empirical relationships have been presented to compute rainfall rate from the measured reflectivity¹, but these relationships vary widely because they are governed by the statistical distribution of drop sizes at the time of measurement. This may not be significant if averaging rainfall over large volumes or long time intervals, but the assumption of an average statistical distribution to compute rainfall rates may result in a large error for applications where data are required for a small volume (for example, the pulse volume of a high-resolution radar) and without long time averages. To overcome this, the essential characteristics of the statistical distribution at a given time and place may be determined from independent measurements of the reflectivity factors obtained using vertical and horizontal polarizations². The technique relies on the fact that large falling raindrops distort to become approximately oblate spheroids, each with its axis of symmetry near vertical, and that the degree of oblateness increases with drop size³. Consequently, the effective radar reflectivity factor measured using horizontal polarization is greater than that measured with vertical polarization.

We report here the first measurements of the two-dimensional spatial distribution of drop size characteristics using switching between polarizations which is sufficiently rapid to be regarded as simultaneous. This technique can substantially reduce errors in radar measurements of rainfall rate in small areas. Furthermore, the technique discriminates between regions of rainfall and dry ice particles. Dual-polarization radar data are expected to be of value in research fields such as radar meteorology, hydrology, cloud physics, radio science, communications engineering and erosion by hydrometeor impact.

The dual-polarization technique

Within a certain range of rain drop sizes, the numerical distribution of rain drops may be represented by an exponential function¹. Such a function may be characterized by two parameters, N_0 and $\bar{D}$, such that:

$$N(D) = N_0 \exp(-D/\bar{D}) \quad (1)$$

where $N(D)$ is the number of drops per unit volume per unit interval for which D is the diameter of the spherical drop with

volume equal to the actual (distorted) drop, and $\bar{D}$ is the mean value of D . Here D and $\bar{D}$ are expressed in mm and $N(D)$ and N_0 in $\text{mm}^{-1} \text{m}^{-3}$. Because of the drop distortion, and direct measurements usually being of drop volumes rather than their diameters, D_0 (mm) is normally used, the diameter of that sphere for which half the volumetric water in the distribution comes from larger drops and half from smaller drops. $D_0 = 3.67 \bar{D}$ as long as $D_0 \ll D_{\text{max}}$, the diameter of the equivolume sphere of the largest drops⁴.

Theoretical relationships based on work by Seliga and Bringi² estimate the parameters N_0 and D_0 from the effective reflectivity factors, $Z_{H,V}$ ($\text{mm}^6 \text{m}^{-3}$), for horizontal and vertical polarizations and the differential reflectivity factor, Z_{DR} , which is defined as

$$Z_{DR} = 10 \log_{10}(Z_H/Z_V) \quad \text{dB} \quad (2)$$

The effective radar reflectivity factors are defined as

$$Z_{H,V} = \frac{\lambda^4 \times 10^{18}}{\pi^5 |K|^2} \int_0^{10 \text{ mm}} \sigma_{H,V}(D) N(D) dD \quad (3)$$

where λ (m) is the wavelength, $K = (n^2 - 1)/(n^2 + 2)$ and n is

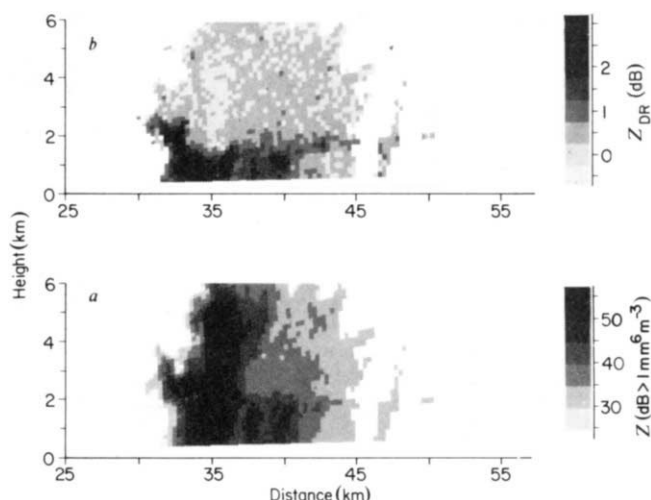


Fig. 1 Vertical scan through raincells at 13.17 UT on 15 August 1978. *a*, Absolute reflectivity factor, Z ; *b*, differential reflectivity, Z_{DR} .

the complex refractive index of water. The backscatter cross-section, $\sigma_{H,V}(m^2)$, for the oblate drops at horizontal and vertical polarizations are obtained from rigorous solutions of scattering from oblate spheroids, and hence there is no assumption of the drops being small compared with the wavelength. For drops of water at the radar wavelength of 10 cm, a reflectivity factor of $1 mm^6 m^{-3}$ corresponds to a scattering cross-section of $3 \times 10^{-12} m^2$ per m^3 of cloud. Usually Z is expressed in dB relative to $1 mm^6 m^{-3}$, that is, $10 \log_{10} Z$. Unless specifically stated, Z_H is used here by convention.

The dual-polarization radar technique has been used to provide accurate measurements of Z_{DR} without excessive averaging of $Z_{H,V}$, which are time varying⁵. As with the present results, the measurements were made using a 10-cm wavelength radar mounted on the SRC's 25-m diameter paraboloidal steerable antenna at Chilbolton, UK. Alternate radar pulses were transmitted on vertical and horizontal polarizations, and examination of the time variation of the two reflectivities sampled from a single radar range gate exhibited certain points crucial to the present measurements. First, the de-correlation time of the measured reflectivity varied between 6 and 45 ms, which was considerably longer than the 1.6 ms taken to measure a pair of reflectivity values. This established that the switching was fast enough for measurement pairs to be sampled before the spatial distribution of rain drops changed significantly. Second, the cross correlation between the two reflectivity records was high, generally about 0.985. This high correlation produced a low standard error of sampling Z_{DR} , which for the system used to collect the present data was 0.1 dB. The corresponding error in Z was 0.75 dB. These random errors result in estimated standard deviations of rainfall rate, for D_0 values of 1 and 2 mm, to be 25 and 20% respectively. Limits to the errors in the calibration of Z and Z_{DR} were estimated to be 1 dB and 0.1 dB, respectively. This would result in a worst case systematic error, for D_0 values of 1 and 2 mm, of 40 and 35% respectively. By future comparison with *in situ* measurements, it is expected that bias due to the system error will be largely eliminated.

In the present study the rainfall rate has been computed using relationships between drop diameters and their fall speeds as obtained by Best⁶. These relationships assume still air, whereas there may be considerable vertical air currents in rain cells. 'Rainfall rate' is a well established characterization of the amount of rain present, and it is used here, even above ground level, without repetition of the phrase 'with respect to a frame of reference travelling at the rate of any vertical air current'.

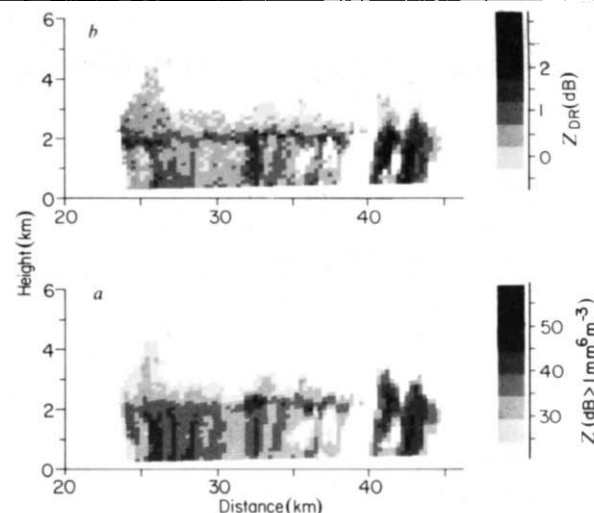


Fig. 3 Vertical scan through raincells at 14.56 UT on 15 August 1978. a, Absolute reflectivity factor, Z ; b, differential reflectivity, Z_{DR} .

Measured spatial distributions of Z and Z_{DR}

As in the system trials, the radar was operated at a frequency of 3,076 MHz and with a peak power of 500 kW. The pulse repetition frequency was 610 Hz, with alternate pulses on vertical and horizontal polarizations. Digitization of data was in 200 steps of 0.3 dB, and averaged in 128 range gates over 0.2 s. The sample gate width of $0.3 \mu s$ was staggered over $2 \mu s$ in $0.5 \mu s$ steps (the radar pulse width) to obtain effective range averaging over a gate width of 300 m. Spatial averaging due to dish movements amounted to one beamwidth for vertical scans (Figs 1–4) and two beamwidths for horizontal scans (Fig. 5). All averaging was of power rather than log power. The data-processing system enabled the received power at each polarization to be processed by the same logarithmic receiver and analogue-to-digital converter.

Figure 1a shows an example of the measured spatial distribution of Z obtained from a vertical scan through a large rain cell. There is a prominent pillar of high reflectivity at 35 km range extending to a height of 6 km, and at ranges of about 38 and 40 km there are small cells of high reflectivity that do not extend above 2 km height. Figure 1b shows the spatial distribution of Z_{DR} sampled at the same time as the data in Fig. 1a. Where the received signal level was less than 5 dB above the receiver noise level, Z_{DR} was set to a 'no data' level (as is also the case for Figs 2–4).

Table 1 Expected characteristics of Z and Z_{DR} at 10-cm wavelength for various hydrometeor types

Hydrometeor type	Z	Z_{DR}	Comments
Rain	High	High	Includes large oblate drops
Drizzle, cloud or fog	Low	Low	Small spherical drops of water and/or small ice particles
Dry snow flakes	Medium-low	Medium-low	Large horizontally oriented low-density aggregates
Sleet/wet snow	High	High	Large oblate horizontal oriented particles
Wet graupel	High	Negative	Large conical vertically oriented particles
Wet hail	High	Variable	Large particles: seldom spheres
Dry hail or other high-density ice particles	Medium	Low	

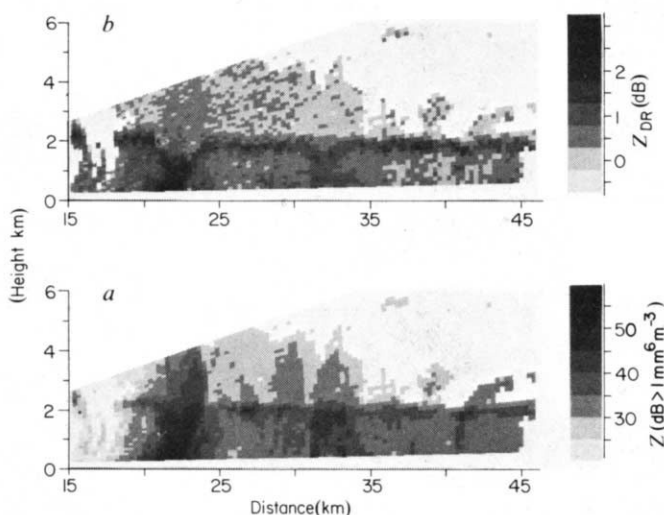


Fig. 2 Vertical scan through raincells at 13.55 UT on 15 August 1978. a, Absolute reflectivity factor, Z ; b, differential reflectivity, Z_{DR} .

One of the most obvious features of Fig. 1a, b is that the column of high reflectivity at 35km range has an associated high value of Z_{DR} (>2 dB) below 2km height, but a value of Z_{DR} of only 0.13 dB (mean, with standard error 0.27 dB) between 2 and 4.5 km height. This abrupt change in Z_{DR} has been seen in many other examples. It always occurs close to the 0°C isotherm, and marks the transition from ice particles to water drops. Unless very large asymmetries are present in low-density ice particles (snow), they will always exhibit a low value of Z_{DR} because of the low refractive index of ice and air mixtures¹. Compact (high-density) ice particles with near-spherical shapes will intrinsically result in low values of Z_{DR} , whereas such particles with irregular shapes are likely to tumble randomly and so also have low Z_{DR} . In the absence of dual-polarization measurements, it would not be known whether the vertical column of high reflectivity in Fig. 1 were

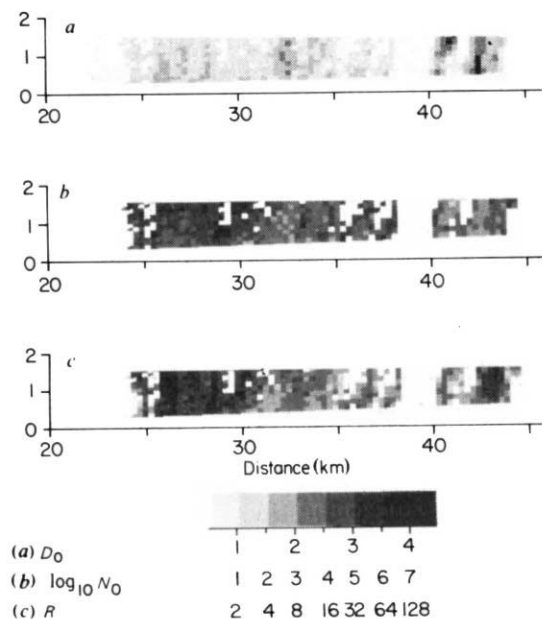


Fig. 4 Spatial distribution of the parameters D_0 (mm)(a); N_0 ($\text{mm}^{-1} \text{m}^{-3}$)(b); R (mm h^{-1})(c) computed from the data shown in Fig. 3, for heights below 1.5 km.

due to ice or water and a convective column containing rain might be assumed. When such convective columns have been observed with the dual-polarization radar, they were clearly distinguishable as having high values of both Z and Z_{DR} throughout their height.

Figure 2 shows data recorded 38 min after that of Fig. 1. There is a 'bright-band' region (of high reflectivity) at 2 km height. The cell at 33 km range has zero Z_{DR} above the bright band, whereas the cell at 22.5 km range, which has much stronger rain below the bright band, has a Z_{DR} of 0.5 dB above the bright band. It is assumed that the depression of the melting region at the centre of these cells, and that seen at 35 km range in Fig. 1, is due to the presence of compact ice particles which take longer to melt and fall faster than snow flakes. When these particles melt they scarcely change in size or fall speed, though the change to water gives the increased Z and Z_{DR} seen in Figs 1 and 2. Indeed this process probably extends to the two smaller and less intense cells to be seen between 37 and 42 km in Fig. 1. It would account for the absence of a height zone of particularly high reflectivity (bright band) near these cells.

In the range 25–30 km in Fig. 2, the largest values of Z_{DR} occur about 200 m below the bright-band height. This may be due to the following series of changes as snow flakes falling below the 0°C isotherm melt slowly. First, water forming on the outer surface of the snow flakes would produce large effective drop diameters and the observed high Z region.

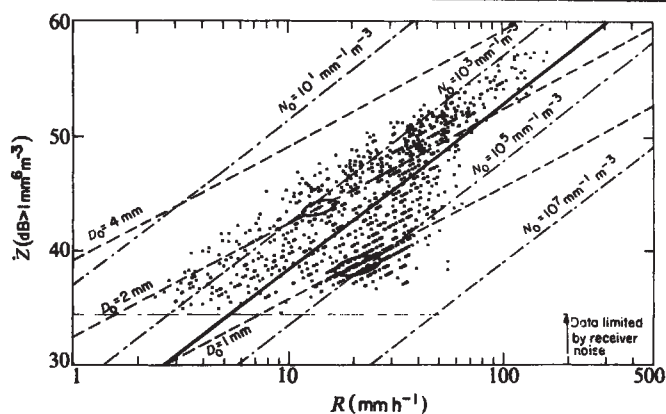


Fig. 5 Distribution of data samples of Z , R , N_0 and D_0 in volume of rain scanned on 15 August 1978 between 13.21 and 13.26 UT—Marshall–Palmer distribution, $N_0 = 8,000 \text{ mm}^{-1} \text{m}^{-3}$.

Subsequently, as the melting continues, the diameters would decrease (causing the observed decrease in Z), but an ice core could maintain a more oblate drop (and higher Z_{DR}) than would be possible with liquid water of the same volume. With further melting, the drop would become less oblate and the Z_{DR} would decrease. Finally, as the melting was completed, the drops would accelerate to their terminal velocity and the concentration of drops would decrease, as would the value of Z .

We shall not speculate further on the distribution of hydrometeors shown in Figs 1 and 2, but from data examined to the time of writing, the expected characteristics for various hydrometeor types are as set out in Table 1.

Rainfall rate and median drop sizes

Figure 3a, b shows data similar to those of Figs 1 and 2, but collected considerably later and in a quite different direction. There is considerable non-uniformity in the spatial distribution of Z and Z_{DR} . Figure 4a, b, c shows the corresponding spatial distribution of D_0 and N_0 of equation (1), and of the rainfall rate, R (mm h^{-1}), computed from Z and Z_{DR} . These are shown only for heights less than 1.5 km as we assume that the hydrometeors were water drops rather than ice particles in this region. Clearly D_0 , N_0 and R all change rapidly with position. This variability may relate to many fields of study. In particular, the attenuation of radio waves due to rainfall may also be computed using N_0 and D_0 (ref. 4), and the variability of this is a current topic of study for both terrestrial and Earth-to-satellite radio paths.

Figure 5 shows values of R computed from Z_H and Z_{DR} plotted against Z (here the mean values of Z_H and Z_V), and superimposed on contours of N_0 and D_0 values. Data were collected up to a height of 1 km and up to 45 km in range whilst the radar was scanning over a width of 15°. Data for which the received signal level was within 15 dB of the receiver noise level, and samples thought to be from ground echoes, have been excluded from Fig. 5. For the lower values of Z in Fig. 5, the computed N_0 varies over the range 10^2 – $3 \times 10^6 \text{ mm}^{-1} \text{m}^{-3}$, and so some data elements have values of N_0 differing by a factor of 300 from that of the much-used distribution of Marshall and Palmer⁷, for which $N_0 = 8,000 \text{ mm}^{-1} \text{m}^{-3}$. The corresponding values of rainfall rates differ from this model by a factor of up to six. For the higher values of Z , there is apparently a much smaller spread of values of N_0 , nearly all of which show lower values than that of Marshall–Palmer. However, all these samples having high Z are due to one concentrated volume of rain, and examination of data similar to those in Fig. 5, but from different rain cells, shows a quite different distribution for high Z values, with N_0 generally greater than the Marshall–Palmer value. Figure 5 also shows experimental sampling error contours at the 1 s.d.

level for D_0 of 1 and 2 mm. Evidently the data show large real differences in drop size characteristics. They also suggest that use of data from a conventional (single-polarization) radar, with an assumed drop-size distribution, may lead to large errors in computation of localized volumes of high rainfall rate.

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An extraterrestrial event at the Cretaceous–Tertiary boundary

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Closely spaced samples from an uninterrupted calcareous pelagic sequence across the Cretaceous–Tertiary boundary reveal that the extinction of planktonic Foraminifera and nannofossils was abrupt without any previous warning in the sedimentary record, and that the moment of extinction was coupled with anomalous trace element enrichments, especially of iridium and osmium. The rarity of these two elements in the crust of the Earth indicates that an extraterrestrial source, such as the impact of a large meteorite may have provided the required amounts of iridium and osmium.

THE Cretaceous–Tertiary boundary seems to be the only major boundary in the stratigraphic record which does not become diffuse when studied in detail^{1,2}. On the contrary, all over the world the boundary can essentially be referred to one single bedding plane, even in the most complete marine sections (Gubbio, Italian Appennines³; Zumaya, Northern Spain^{4,5}; El Kef, Northern Tunisia⁶; and many DSDP holes^{7,8}). Planktonic Foraminifera occur abundantly in these sections and thus provide one of the best available ways of following the extinctions at the end of the Cretaceous¹. This extinction and subsequent resurrection has been studied in detail in the unusually complete and thick calcareous pelagic section of Caravaca in South-east Spain⁹. Here, the topmost Maastrichtian and lowermost Palaeocene biozones (the *Micula prinsii* Zone, P. Nielsen, personal communication and the '*Globigerina*' *eugubina* Zone respectively) have been demonstrated. A 10-cm thick 'intermediate' bed also occurs between these zones characterized by a threefold increase of clay minerals and a peculiar indigenous fauna (J.S. in preparation). Information from the other sections mentioned above, although less complete, is compatible (Fig. 1). In the >100-m thick youngest Maastrichtian marls of the Caravaca section the rich, tropical association of planktonic Foraminifera and nannofossils^{9,10} shows no significant changes up to the very last centimetre; here almost the entire association disappears within 0–5 mm. At a Maastrichtian sedimentation rate of 7 ± 3 cm kyr⁻¹ (Fig. 1) this implies that the extinction

took place within ~200 yr (ref. 1).

The impoverished association in the subsequent 10 cm intermediate bed is dominated by benthonic Foraminifera and relicts of the Cretaceous planktonic fauna, like *Hedbergella monmouthensis* (Olsson), *Globigerinelloides aspera* (Ehrenberg), *G. messinae* (Brönnimann) and *Guembelitra cretacea* (Cushman). The amazingly sharp limits of this level are apparently preserved owing to a temporarily decreased rate of burrowing, because all other sediments in the section are strongly bioturbated.

At the top of this intermediate bed the new Tertiary planktonic fauna suddenly appears in great numbers. Accompanied by the sole survivor of the terminal Cretaceous 'holocaust', *Guembelitra cretacea*, this new fauna diversifies, and shows a rapid succession of different dominant species in the lowermost 50 cm of the Palaeocene, representing the entire '*G.*' *eugubina* Zone¹ (Fig. 1). Hereafter the facies becomes similar to that of the Upper Cretaceous, exhibiting the same, normal, slow evolutionary changes in fauna and flora.

Analyses

To obtain more information about the extinction event (to sort out some of the current extinction models²), trace elements have been analysed by instrumental neutron activation (NAA) on 100 bulk samples closely spaced around the boundary: 17 elements were detected throughout the section and 10 more

Table 1 Anomalous trace element enrichments in the lowermost Tertiary of the Barranco del Gredero, Caravaca, South-east Spain

	Upper Cretaceous				Intermediate bed				Lower Palaeocene			
	$\bar{X}^*$	$s^\dagger$	$\overline{c.v.}$	n	$\bar{X}$	$\overline{c.v.}$	n	$\bar{X}$	s	$\overline{c.v.}$	n	
Ir	0.13	p.p.b.	—	2	25.5	p.p.b.	—	0.34	p.p.b.	—	1	
Os	0.08	p.p.b.	—	2	16.1	p.p.b.	—	0.41	p.p.b.	—	1	
Ni	24.2	p.p.m.	—	2	1065.7	p.p.m.	4	40.6	p.p.m.	14	8	
Co	9.06	p.p.m.	2.8	28	270	p.p.m.	3	5.59	p.p.m.	2.5	65	
Cr	56.9	p.p.m.	20	28	499	p.p.m.	2.5	69.6	p.p.m.	19.3	63	
As	2.07	p.p.m.	1.23	16	225.8	p.p.m.	12	1.66	p.p.m.	1.5	31	
Sb	0.35	p.p.m.	0.09	19	7.01	p.p.m.	10	0.23	p.p.m.	0.08	24	
Se	0.073	p.p.m.	—	2	5	p.p.m.	—	0.138	p.p.m.	—	1	

* Sample mean.

† Sample standard deviation.

‡ Mean coefficient of variation of the analytical error (%).

§ Number of analyses.

Analytical error for Ir, Os, Ni and Se for high values better than 2%, for low values better than 10%.

Ca	-0.94																	
Sc	0.97	-0.95																
V	0.96	-0.94	0.96															
Cr	0.86	-0.86	0.89	0.91														
Mn	-0.61	0.54	-0.59	-0.56	-0.5													
Fe	0.96	-0.94	0.97	0.94	0.85	-0.6												
Co	0.87	-0.83	0.84	0.82	0.75	-0.66	0.81											
Rb	0.82	-0.82	0.81	0.74	0.63	-0.61	0.85	0.7										
Cs	0.72	-0.73	0.73	0.63	0.51	-0.51	0.77	0.57	0.96									
La	0.6	-0.67	0.71	0.66	0.72	-0.21	0.67	0.42	0.53	0.49								
Ce	0.82	-0.84	0.89	0.8	0.74	-0.42	0.89	0.63	0.79	0.78	0.76							
Sm	0.52	-0.57	0.61	0.56	0.53	-0.16	0.58	0.39	0.43	0.35	0.86	0.58						
Eu	0.45	-0.54	0.58	0.51	0.59	-0.11	0.54	0.33	0.4	0.47	0.75	0.73	0.46					
Yb	0.14	-0.26	0.28	0.23	0.33	-0.12	0.23	0.05	0.16	0.21	0.76	0.43	0.55	0.75				
Hf	0.88	-0.86	0.87	0.82	0.77	-0.56	0.88	0.8	0.88	0.77	0.67	0.77	0.59	0.46	0.27			
Th	0.96	-0.94	0.97	0.92	0.83	-0.6	0.98	0.81	0.9	0.83	0.69	0.89	0.59	0.56	0.27	0.92		
Insoluble residue	0.88	-0.87	0.91	0.85	0.78	-0.47	0.93	0.7	0.83	0.76	0.69	0.87	0.6	0.54	0.24	0.83	0.92	
	Al	Ca	Sc	V	Cr	Mn	Fe	Co	Rb	Cs	La	Ce	Sm	Eu	Yb	Hf	Th	

Fig. 1 The Cretaceous-Tertiary boundary interval in the Barranco del Gredero, Caravaca, South-east Spain. A, *Micula prinsii* Zone; B, 'intermediate' bed; C, *Globigerina eugubina* Zone; D, *Globigerina pseudobulloides* Zone.

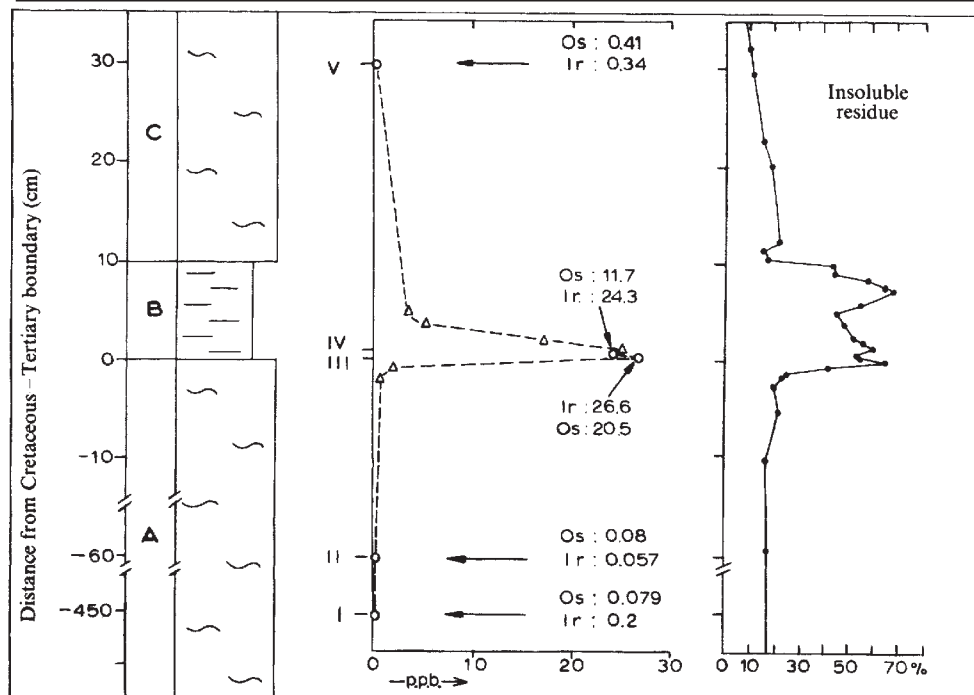


Fig. 2 Iridium, osmium and insoluble residue data from the Cretaceous-Tertiary boundary in the Barranco del Gredero. The iridium data are plotted. $\circ$, I-V Samples analysed at the Institute for Nuclear Sciences, Gent, Belgium. Δ , Preliminary Ir analyses, communicated by W. Alvarez. A, Micula prinsii Zone; B, 'intermediate' bed; C, 'Globigerina' eugubina Zone.

that none of the existing models is entirely satisfactory, but that only a very short event, which a great part of the biosphere could not tolerate, may explain the known facts. The above mentioned points support this view.

Current extinction models may be classified in two ways; the gradual versus the catastrophic or the terrestrial as opposed to the extraterrestrial models. Most terrestrial models (nutrient depletion¹⁷, climatic deterioration¹⁸, rise in CCD¹⁹, CO₂-O₂ imbalance²⁰, secular variations²¹ and increase in volcanism²²) are essentially gradual and should leave traces in the sediment of the imminent extinctions. None has been found yet. Reports of gradual extinction^{4,19} lose their credibility on more detailed inspection¹.

There remain the catastrophic models which do not have a pre-extinction signal. One such model is the recently advocated 'Arctic flushing' of cold brackish water over the ocean surface^{7,23}. In this way, however, the excess of iridium and osmium cannot be explained. Extraterrestrial influences (giant solar flare²⁴, supernova^{2,18,25} or asteroid impact²⁶⁻²⁸) on the other hand are geologically speaking instantaneous and in particular the latter explains the high amounts of iridium and osmium. The frequency of disastrous impacts on the Earth or the explosion of a nearby supernova have been estimated; once every 100 Myr an impact of an asteroid or comet nucleus 10-30 km in diameter²⁶⁻²⁸ or every 70 Myr a supernova at a distance of 50 light yr occurs^{2,25}. A typical iron meteorite is $\sim 2 \times 10^4 - 4 \times 10^5$ times, and a chondrite $\sim 6 \times 10^3 - 2 \times 10^4$ times, enriched in Ir and Os relative to the crust of the Earth and when such body vaporizes on impact, or is partly broken up on entry in the atmosphere, it may deliver overdoses of Ir and Os over a large area; if we assume the Caravaca values $-3 \times 10^{-8} \text{ g cm}^{-2}$ — dispersed over the whole world and an iridium content of $5 \times 10^{-7} \text{ g per g}$ in a carbonaceous chondrite it may imply an impacting body of about 5 km in diameter. Geological evidence for an impact, such as a crater larger than 150 km in diameter or impact triggered sediment at the boundary have not yet been found.

The expanding shell of a supernova explosion could sweep up amounts of Ir and Os from interstellar matter.

The maximum amount that can be swept up by a supernova at 50 light yr distance is only the equivalent of the Os and Ir content of a chondrite with a diameter of 60 m. Hence it falls short by a factor of 10^6 with respect to the required amounts (E. P. J. van den Heuvel, personal communication). Also the ratio of the stable osmium isotopes $^{184}\text{Os}/^{190}\text{Os}$ is very similar to Solar System

ratios¹². It would be surprising if the isotopic composition in interstellar matter is within 0.1% of Solar System ratios.

Conclusions

The present evidence favours an extraterrestrial cause for the extinctions at the end of the Cretaceous. The impact of an asteroid or comet 5-15 km in diameter is the most attractive. The consequences of such an event are still poorly understood and need further investigation, as not all the facts fit this model easily.

A crucial point in the model is the near synchronous extinction of all organisms that did not cross the Cretaceous-Tertiary boundary, and the independence of the event from the known normal environmental processes going on in the latest Cretaceous.

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Terrestrial catastrophe caused by cometary impact at the end of Cretaceous

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Evidence is presented indicating that the extinction, at the end of the Cretaceous, of large terrestrial animals was caused by atmospheric heating during a cometary impact and that the extinction of calcareous marine plankton was a consequence of poisoning by cyanide released by the fallen comet and of a catastrophic rise in calcite-compensation depth in the oceans after the detoxification of the cyanide.

MASS extinction at the end of Cretaceous¹ has been related to the impact of meteorites², asteroids³, or comets^{4,5}. The two groups of organisms most affected are large terrestrial animals and calcareous marine plankton, especially those in tropical waters. Here it is suggested that cometary impact was the major cause of the extinction of these two groups.

Catastrophic nature of the terminal Cretaceous event

High-resolution stratigraphy indicates the very short duration of the terminal Cretaceous event⁶⁻⁹. The biological changes of the marine-planktonic world across the boundary could be separated into three phases: (1) the mass extinction of a rich marine-planktonic fauna of latest Cretaceous age; (2) the predominance of small foraminifera and some special nannofossils which might be surviving species from the Cretaceous⁷; (3) the appearance and evolution of robust Tertiary species.

The first phase is recorded by an iridium-rich clay or marly layer some 1 cm thick in Italy¹⁰, and 2–5 mm thick in Spain⁹. The second phase is recorded by the *Globigerina eugubina* zone^{8,9}. The third act is represented by the higher Danian microfauna and nannofossil stages. Using sedimentation rate as a basis, the duration of the terminal Cretaceous event which caused the mass extinction was estimated to be <10,000 yr (ref. 11). In fact, the span of time could be <50 yr, if the 'iridium-spike' of Spain represents the record of that event⁹. Large terrestrial animals may have become extinct at the same time as the marine plankton¹² although this is still uncertain.

Although the terminal Cretaceous event lasted a very short time, the damage was extensive¹⁴⁻¹⁶ and the destruction was selective. Evolutionary changes of terrestrial plants were gradual¹⁷ and small animals living in freshwater were virtually unaffected by the extinction¹⁶. However, large terrestrial animals heavier than 25 kg did not survive the catastrophe. In the marine realms, calcareous plankton was hard hit, with some groups (for example, planktonic foraminifers and nannoplankton) being virtually wiped out¹⁴.

The extinction seemed to have hit organisms at different stages of their evolutionary development¹⁵. Also there seems to have been palaeogeographical selection; virtually all Cretaceous organisms which became extinct near a major peak in their evolutionary development lived predominantly in or very near to the equatorial Tethys¹⁸, and their vacant tropical niches were eventually occupied by new Tertiary species, which had evolved from survived stocks of eurytopic temperate or cosmopolitan faunas¹⁸. Finally, selectivity might have been an expression of resistance to environmental stress. For example, the sparse nannofloras surviving the terminal Cretaceous event were dominated by *Braarudosphaera* and *Thorascosphaera*, both of which tolerate, or even prefer, conditions adverse for the growth of other calcareous nannoplankton in the present oceans¹⁴.

The fossil records register a catastrophic revolution of the biosphere. The almost invariable presence of a clay layer or a

hardground boundary at the Cretaceous-Tertiary is evidence of a revolutionary change in the entire hydrosphere, manifested by a rise of the CCD (calcite compensation depth), which in turn, was caused by an increase of dissolved CO₂ in seawater^{13,14,19}.

Studies of trace elements in the clay-rich layer just above the contact indicates an unusual concentration of iridium^{9,10}. The isotopic composition of Ir indicates extra-terrestrial input from a source within the Solar System¹⁰. Also enriched are Co, Ni, Zn, As and Sb⁹.

The clay-rich layer is absent at the Cretaceous-Tertiary boundary in some deep-sea drilling holes, for example, DSDP Holes 356, 374^{20,21}, where biostratigraphical analyses invariably indicate, a depositional hiatus (refs 13, 21 and K. P. Nielson, personal communication). At some localities the contact is represented by a hardground²² indicating widespread erosion or non-deposition as a consequence of bottom-current activities²¹.

The climatic changes during the late Cretaceous were gradual^{17,23-27}. However, isotopic analyses of deep-sea drilling cores during the past few years indicate remarkable temperature difference across the Cretaceous-Tertiary boundary²⁶. An increase of 1–5 °C of both bottom and surface temperatures has been recorded by oxygen-isotope data from widely scattered localities²⁶⁻²⁹.

Analyses of carbon isotopes of sediments across the boundary suggest a remarkable change in the chemistry of the ocean water associated with the terminal Cretaceous event. A decrease of ¹³C of 1–3‰ has been reported²⁵⁻³⁰ equivalent to a change produced if the whole of the terrestrial biosphere were put into the ocean²⁵!

Frequency and consequences of comet collision

Impact craters are not uncommon on the surface of the Earth³¹. Based on existing craters, Hughes³² obtained an estimate of the rate of cratering, which can be represented by

$$\phi = \frac{1}{1,400D^2}$$

where ϕ is the rate on Earth (yr⁻¹) and D is the diameter of the crater (km). This estimates that a small crater of 200 m may be created every 350 yr, and a large crater of 100 km every 14 Myr. Based on tektite or crater ages, Napier and Clube³ noted that large (>50 km) impact-cratering took place during the Pleistocene (1 Myr), middle Miocene (15 Myr), late Oligocene (28 Myr), early Oligocene (35 Myr), late Eocene (38 Myr), beginning of Jurassic (183 Myr), middle Triassic (210 Myr), and late Devonian (365 Myr). Considering that almost three-quarters of the Earth's surface lie under water, one can expect oceanic craters to have largely escaped detection. Nevertheless, the statistics indicate that there should have been at least one crater bigger than 500 km during the 600 Myr of the Phanerozoic.

The energy E generated by the craters has been variously

estimated^{4,5,33,34} depending on the size of the object and its collision velocity. Assuming an impact speed of 24.6 km s^{-1} , Napier and Clube⁵ obtained E values ranging from 0.3 to $150 \times 10^{30} \text{ erg}$, associated with 80 – 500 km cratering which could be produced by planetesimals 4 – 31 km in diameter. Urey⁴ used a collision velocity of 45 km s^{-1} for a comet with a mass of 10^{18} g , which is the estimated size of the Halley's comet, and found that the impact energy is 10^{31} erg , or $250 \times 10^6 \text{ Mton}$. If all energy were absorbed by the atmosphere, the air temperature would rise by 190°C . If all energy were absorbed by the oceans, the elevation of average ocean temperature would be 0.175°C . If all the energy were used to excavate a crater, a mass of dust $3 \times 10^{19} \text{ g}$ could be thrown up to encircle the Earth.

Discussing Moon cratering, Gault *et al.*³⁵ indicated that a third of the kinetic energy is converted to heat, which is manifested in the melting and/or vaporization of both the impacting and the impacted material. However, comets falling into the ocean may heat up both the atmosphere and hydrosphere by friction. The atmospheric heating caused by a small comet may absorb the greater share of the kinetic energy. The Tunguska explosion on 30 June 1908 may have been caused by a small comet with a mass of $5 \times 10^{10} \text{ g}$, and the comet may have exploded and disintegrated in the air so that numerous craters 50 – 200 m in diameter, instead of one large crater, were found in the impact area^{2,36}. The heat was so intense that it killed all animal life within $1,000 \text{ km}^2$ (ref. 3). Aside from the kinetic energy of $5 \times 10^{23} \text{ erg}$, Hughes³⁶ suggested that a nuclear explosion may have been triggered by neutron production as the heated comet sped through the atmosphere; he thus offered an explanation for the ^{14}C anomaly of 1909. For a very large comet which might generate 10^{31} erg , the fraction of kinetic energy expended to heat the atmosphere depends on the entry angles and other factors. It is unlikely that the whole atmosphere could be heated to 190°C as Urey suggested for the extreme case of total dissipation by atmospheric heating. On the other hand, the temperature produced by a speeding comet may have been so high locally that it could produce nuclear or even thermonuclear reactions (as Hughes suggested) in addition to chemical explosions. With the possible addition of chemical and nuclear energy, it is difficult to estimate the possible heating of the atmosphere. It seems, however, that a 10 – 20°C increase of air temperature is possible. The heating of a large comet falling through a 5-km deep ocean can be calculated on the basis of estimating hydrodynamic resistance. Using a resistance coefficient of 0.001 typical of motions with a very large Reynolds number³⁷, I obtained a value of 10^{30} erg , or about 10% of the total kinetic energy for a fallen comet with 30 km diameter, 10^{18} g mass, and 45 km s^{-1} speed. These estimates suggest that the bulk of the kinetic energy would be expended after impact, causing cratering and melting of substratum, which should become an additional source of energy to raise the temperature of the ocean further.

Chemical consequences

While the physical consequences of a large cometary collision are impressive, the chemical consequences are commonly neglected because we know little about the composition of comets. There are still debates over whether comets are dirty snowballs or dust swarms³⁸, although the first, also known as the icy conglomerate model first formalized by Whipple, is being favoured^{38–40}. The model assumes that the nucleus of comets is an icy solid body containing chemical compounds which sublime and dissociate to provide the radicals and simple molecules that have been observed in cometary spectra^{40,41}. Optical spectra showed the presence of CN , CO^+ , CH , NH , QH , and so on⁴². When Comet Kohoutek came near the Earth in 1973, hydrogen cyanide and methyl cyanide were detected 'at a reasonable abundance'^{40–42}. If the concentration were 10% the amount of cyanides dissolved from a 10^{18} g of comet, and evenly distributed in ocean water would give a concentration of 0.1 p.p.m. . However, the concentration would be 3 p.p.m. if the dissolved substance was concentrated in the upper 100 m only, and even

more if the poison was carried by currents and thus contained within the moving surface water mass.

CO_2 and/or CO are probably also present in the nucleus of a comet. The ocean has $130 \times 10^{18} \text{ g}$ of dissolved carbonates⁴⁴ mainly as HCO_3^- . Deeper ocean water is undersaturated due to its higher dissolved CO_2 and lower pH . However, the surface seawater is supersaturated because of the high pH related to disturbed buffering caused by photosynthesis. The annual CO_2 extracted from the ocean by marine plankton is $\sim 0.4 \times 10^{18} \text{ g}$, which is the same order of magnitude as the probable mass of CO_2 in a 10^{18} g comet. The sudden dissolution of CO_2 from a large fallen comet can thus greatly disturb the carbonate equilibrium, so that surface waters, at least locally, may become temporarily undersaturated.

A large input of cometary carbon should also significantly alter the isotopic composition of calcareous sediments in the ocean. The isotopic composition of carbon for meteorites and for the Earth's mantle has about the same composition of -6 to -7% ^{13}C . However, the carbonate in meteorite and the carbon in chondrite have very heavy ^{13}C , with values up to more than $+50\%$ (ref. 45). As the CO_2 in the nucleus of a comet may have represented a distilled fraction, it should have an isotopic composition much lighter than that of the average meteorite. Assuming a cometary $\delta^{13}\text{C}$ of -25% , a -1.5% carbon-excision in dissolved carbonate can be caused by the fall of a 10^{18} g comet of which carbon atoms constitute a quarter of its mass.

Comet impact at end of Cretaceous

Probability calculations indicate that at least one large comet with a mass of 10^{18} g and impact energy of 10^{31} erg should have hit the Earth during the Phanerozoic. The nature and severity of the terminal Cretaceous catastrophe suggest that such a comet hit the Earth at that time. The comet must have fallen into the ocean so that there would be no evidence on land of a crater of this age.

The preceding analysis showed that a cometary impact may kill by three different means: large land animals could be killed by atmospheric heating, marine organisms could be killed by cyanide poisoning, calcareous marine plankton might further suffer because of a catastrophic rise of the CCD of the oceans.

If the comet fell into the ocean, the dissolution of its nucleus should have released a large quantity of HCN , CH_3CN , CO_2 , and possibly also CO . The dissolved constituents should ascend with the warm water mass heated by the fallen comet, and should find their way into a surface current system and remain relatively concentrated there. Cyanide is a powerful inhibitor, arresting cellular respiration by inactivating metallo-enzymes in the respiratory process⁴⁶. As this process is fundamental to all living organisms, cyanide dissolved in sufficient concentration could kill all kinds of marine organisms, including nannoplankton. The cyanides could be reduced through oxidation into CO_2 (ref. 46). This CO_2 added to the extraterrestrial CO_2 brought in by the comet would increase the dissolving power of seawater. The mass-mortality of phytoplanktons would further upset the CO_2 budget with a consequent catastrophic rise of CCD, leading to a virtual extinction of the calcareous planktonic life. Furthermore, phytoplankton formed the basis of the food chain for higher organisms, so that their mass mortality should cause starvation of those marine organisms¹⁹ that had escaped cyanide poisoning. Detoxification of cyanide also produced nitrates. Experiments with living nannoplankton indicated that they would not secrete calcareous skeletons in nitrate-rich waters (B. Funnell, personal communication). Such nannoplankton would leave no fossil record, but may have evolved into the Tertiary forms which did secrete skeletons when conditions returned to the normal.

Surface currents would converge eventually into the Equatorial Current, where the greatest contamination would occur. Consequently, the catastrophe would hit hardest those organisms which inhabited the tropics. Some organisms living in the gyres of the middle latitude would escape extinction, not

only because they were more tolerant of stress conditions, but also because the gyres were regions bypassed by polluted surface currents. Note, for example, that the nannoplankton *Braarudosphaera* is not only a tolerant species, but its modern representatives also have a population in the gyre of the Sargasso Sea⁴⁷. Large parts of the deep-sea bottom may also have been spared lethal contamination, so that deep marine benthic communities mostly survived the catastrophe.

On land, the large vertebrates would perish under an almost instantaneous thermal stress^{2,16,24}, but small or aquatic animals would largely survive. Laubenfels² suggested that turtles may have escaped the heat wave by diving under water for hours while holding their breath, and that crocodiles preserved their species because they had buried their eggs in mud. Because the comet fell in the oceans, temperature anywhere on land may not have been sufficiently high to cause global forest fires. In any case, land vegetations could have escaped extinction as new seeds could sprout even after a pyric catastrophe.

The palaeo-oceanography after the comet fell must have been extraordinary. The increase of dissolved CO₂, the mass extinction of phytoplanktons, and the consequent pH changes may have raised the CCD to a level near or within the photic zone. Consequently, the sediment underlying the oldest fossiliferous Tertiary beds is commonly a clay or clay-rich. The input of trace elements from the fallen comet would have contributed to the unusual concentration of Ir, As, Ni and Co in this clay-lamina^{9,10}. Meanwhile, the presence of molten rock material in an impact crater under the ocean should generate thermal stress and promote active bottom-circulation in the deep sea. Consequently, a depositional hiatus other than dissolution-unconformity has been noted at many deep-sea drilling sites across the Cretaceous-Tertiary boundary. The input of converted kinetic energy, possibly supplemented by the chemical and nuclear energies, would cause the rise in ocean temperature, as manifested by the $\delta^{18}\text{O}$ shift in the oldest Tertiary sediments. The input of light extraterrestrial carbon would contribute to the accompanying carbon shift.

The climatic implication of the postulated cratering is not clear. Urey⁴ as well as Napier and Clube⁵ estimated that the ejectas excavated by a large fallen comet may equal, or even exceed, the mass of the comet, and much of the fine dust might reach the stratosphere to block out solar radiation, thus causing glaciation. Indeed, the timing of drastic cooling near the end of Eocene and in middle Miocene⁴⁸ coincided remarkably with that of the Popigai cratering (100 km diameter) in USSR at

38 Myr and the cratering which produced the Moldavites at 15 Myr (ref. 5). The end of the Cretaceous has been considered the beginning of a general cooling on the Earth¹⁷. Isotopic shift did show cooling during the first few million years of the Palaeocene following the 'heating spike' (ref. 27). However, the temperature was apparently lowered to that prevailing before the catastrophe. The emerging deep-sea record indicates that the first Tertiary cooling responsible for the initiation of Antarctic glaciation came much later, at the end of Eocene⁴⁸, or the time of Popigai cratering. The different climatic effects of cratering may be accounted for by the postulate that the terminal Cretaceous comet fell into the ocean: much of the dust-sized ejectas were trapped by the ocean and did not reach the stratosphere, in contrast with the continental landing of a comet at the end of Eocene.

Conclusions

We may never find the crater, because much of the Mesozoic ocean floor has disappeared by subduction during the past 65 Myr. However, if the crater has not yet been subducted, systematic searches should locate such a large feature. The crater should have a diameter of a few hundred kilometres. Furthermore, the cratering should have created an ejecta plume, namely rock-lava steam mixture rising vertically above the crater like a volcanic eruption⁴⁹. Collapsing under its own potential, the mixture should have developed into a *nuée ardente* or base-surge^{50,51}. The mass flow at high speed should have eroded the ocean floor and formed channels. All those geomorphic and depositional features can be detected by seismic profiling.

The cratering should have produced earthquake waves and tsunami waves which would cause subaqueous slumping and turbidite deposition. We could search for the crater through a systematic recording of the size and distribution-density of such mass-flow deposits. Likewise, we could study the palaeotemperature distribution to trace the geographical origin of the anomaly. We could study the hiatus-distribution to trace the source of the stress that induced the bottom circulations. We could refine our study of geographical patterns of mass extinction. A careful examination of the existing geological records may tell us whether the crater is still to be found or if it ever existed.

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Bulk attenuation in the Earth and viscosity of the core

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Recent studies indicate that bulk absorption is required somewhere in the Earth to explain the damping of the radial modes of free oscillation. This excess absorption, if attributed to bulk viscosity in the core, requires a value of ~ 500 P. This is theoretically reasonable and may be due to structural or concentration fluctuations. The shear viscosity is at least several orders of magnitude smaller.

ALTHOUGH most of the seismic attenuation in the Earth can be attributed to losses in shear¹ the damping of the radial modes suggest that bulk losses occur somewhere in the mantle or core²⁻⁴. If all losses were in shear value the Q value of the fundamental radial mode, ${}_0S_0$, would be $>7,000$. Several recently determined values are $\sim 4,100$. The other radial modes and compressional spheroidal overtones also have high Q values but they are more sensitive to shear attenuation than ${}_0S_0$, the most nearly pure compressional mode. Discussions of bulk, or volume, attenuation, therefore, must rely mainly on this mode.

The mode ${}_0S_0$ has only been observed infrequently and the reported values for Q have a wide variation⁵. Narrow band filtering experiments indicate that its amplitude does not die off uniformly with time after an earthquake (H. Kanamori and A. Dziewonski, personal communications). During the first few days there seems to be interference⁴ with adjacent, stronger excited modes, such as ${}_0S_1$. The decay after about 2 weeks is less rapid than the initial decay. This could be a problem of the lower signal-to-noise ratio in the later portions of the record or a re-excitation by long-period aftershocks. Some recent results are given in Table 1. Note that relatively high values are obtained if the early part or the late parts of the record are included. The first four entries in the table are consistent, within the assigned errors, and give an average value of 4,590 with an uncertainty of $\sim 11\%$. This includes two multiple station analyses. An analysis by Kanamori (personal communication) for the period 1–25 days after the Indonesian event gives a Q of 5,425. The first 2 weeks of data, however, fall in the above range. For present purposes we assume that Q of ${}_0S_0$ is $\leq 4,800$ and investigate the implication. If later studies establish a higher value, then the viscosities inferred in this article will be upper limits.

Sailor and Dziewonski⁴ attempted to fit the radial modes by inserting a low Q_k region in the upper mantle. The radial mode ${}_0S_0$, however, has little energy in the upper mantle and is most sensitive to properties of the lower mantle and outer core. Anderson and Hart³ satisfied the ${}_0S_0$ data by assuming a Q_k of 10^6 in the outer core; their models also have a low Q_k , 426–847, in the inner core. These values were based on body wave and inner core shear mode data, as well as on the radial modes.

The problem is not unique as there is little resolving power in the available compressional or high- Q modes. We have found that a uniform Q_k of $\sim 10^4$ throughout the Earth also satisfies these data. If only the core contributes then a slightly smaller value for this region is implied. Body wave studies^{6,7} of the outer core give values for Q_p ranging from 10^2 to 10^4 . This suggests that the core may contribute significantly to the observed attenuation. Furthermore, bulk attenuation is well documented in fluids and, in particular, in liquid metals. Because of the dominance of shear losses in the mantle it will be difficult to determine the bulk contribution, if any, of this region. In this article we estimate the bulk viscosity of the core from the

damping of the radial modes and then investigate if this is a reasonable number for liquid iron at core conditions.

Models

We have investigated a large number of models to learn more about possible distributions of bulk attenuation in the Earth. These included constant Q_k and frequency-dependent Q_k models, all of which satisfy the shear sensitive spheroidal and toroidal mode data. The excess absorption is attributed to bulk losses in pure compression. Some of these models and the resulting radial mode Q spectra are given in Table 2. SL8 is from Anderson and Hart³ and is a frequency-independent Q structure. The others are a result of the present study and, at low frequencies, have $Q_k \sim \omega^{-1}$ as appropriate for a relaxation mechanism. The values given are for a period of 1,000 s. The radial mode data and results for three models are shown in Fig. 1.

The value of $\sim 7,000$ for Q (${}_0S_0$) which is obtained for Earth models exhibiting only shear losses can be reduced to a value near 4,200 by introducing a Q_k of $\sim 10^4$ distributed uniformly throughout the mantle and core. If the bulk losses occur only in the core an average Q_k of $\sim 2 \times 10^3$ in this region is required.

As

$$Q_k^{-1} = (2\pi f / \rho c^2) \eta_v \quad (1)$$

for fluids this gives an effective bulk viscosity, η_v , for the core of ~ 500 P; this is an average value. ρ is the density; c , the velocity of sound; and $f = \omega / 2\pi$ is the frequency. Pressure is likely to increase viscosity so that smaller values may be maintained at the top of the core. There is no detailed information about the variation of Q_k with depth but we know that the attenuation of P waves is much greater in the inner core than in the outer core. This suggests that the relaxation time in the inner core is closer to seismic periods than is the case in the outer core, and that the relaxation time increases with depth. This implies an increase in viscosity with depth. Note that previous investigators have proposed that the inner core is a highly viscous fluid rather than a crystalline solid⁸.

Table 1 Q of ${}_0S_0$

Q	Error (%)	No. of stations	Days	Earthquake	Ref.
4,000	5	1	5–15	Alaska	4
4,100	26	5	2–24	Indonesia	22
4,589	4	7	2–16	Indonesia	*
Shorter times included					
5,663	9	1	1–17	Alaska	4
6,687	13	2	0–25	Indonesia	5
Longer times included					
5,527	2	7	2–45	Indonesia	*

* A. Dziewonski (personal communication).

Physics of attenuation in fluids

We will first consider sources of attenuation in the outer core. The absorption of sound in a liquid at low frequencies is given by

$$\alpha/f^2 = (2\pi^2/\rho c^3) \{ \frac{4}{3}\eta_s + (c^2\beta^2\theta T/C_p^2) + \eta_v \}$$

where α is the spatial attenuation coefficient, β the thermal expansion coefficient, θ the thermal conductivity, C_p the specific heat, η_s the shear viscosity, η_v the volume viscosity, and T the absolute temperature. The first two terms on the right-hand side represent the classical Stokes–Kirchoff absorption due to shear stresses and thermal conductivity. The first term is important in the attenuation of longitudinal waves in liquid metals, but is absent for strains involving pure compression. The second term is important at laboratory frequencies but is negligible at core conditions and seismic frequencies. The remaining term is called the excess absorption and is due to bulk or volume viscosity. Both η_s and η_v contribute to damping of longitudinal waves in fluids but only η_v would contribute to the purely compressional strains which dominate the fundamental radial mode. In liquid metals at low pressure η_v is similar in magnitude to η_s (ref. 9).

Several mechanisms contribute to bulk viscosity in simple fluids. One is structural relaxation due to perturbations in the short-range order caused by the sound wave. Another is due to concentration fluctuations in alloy systems. These are comparable in magnitude in laboratory conditions but have quite different temperature, and possibly pressure dependencies. The dominant mechanism may, therefore, be different at core conditions from that in laboratory conditions. A high bulk viscosity at conditions prevailing in the core is not necessarily inconsistent with a much lower shear viscosity. The characteristic times for shear and structural relaxation are likely to be similar as they are both controlled by short range diffusion. The relaxation time due to concentration fluctuations or chemical reactions may be quite different.

Longitudinal waves in liquid metals typically are within an order of magnitude of

$$Q = 10^{11}/f$$

which in normal conditions is composed of three roughly equal parts due to shear viscosity, thermal conductivity and bulk viscosity, or excess sound absorption⁹. The attenuation in liquid metals in laboratory conditions would be quite negligible at seismic frequencies. However, the effects of pressure and alloying lengthen the characteristic relaxation times.

Bulk viscosity of molten iron

The bulk viscosity of a pure liquid metal is due to structural rearrangement as there are no internal degrees of freedom. Two-state theories have been developed to explain structural relaxation associated with pressure changes. The assumption is that a liquid is a mixture of two structural states with differing mole volumes and energies.

We use the two-state theory of Herzfeld and Litovitz¹⁰ and Flinn *et al.*⁹ to estimate the structural volume viscosity of iron just above the melting point, T_m , and its relation to the shear viscosity;

$$\eta_v = \frac{x_1 x_2 V}{RT_m} \left\{ \frac{\Delta V}{V} - \frac{\beta \Delta H}{C_p} \right\}^2 \tau K^2$$

and

$$\eta_v/\eta_s = \frac{(2n-12)(12-n)}{n^2} \left\{ \frac{VK}{RT_m} \right\}^2 \left\{ \frac{\Delta V}{V} - \frac{\beta \Delta H}{C_p} \right\}^2$$

$$x_2 = (12-n)/n = 1-x_1$$

where x_1 and x_2 are the mole fractions of the two states which differ by ΔV and ΔH in molar volume and enthalpy, and n is the coordination number. For liquid iron at low pressure we take $n = 9$; $V = 8 \text{ cm}^3 \text{ mol}^{-1}$; $\Delta V/V = 0.1$; $\Delta H/RT_m = 3$; the bulk modulus, $K = 1.3 \text{ Mbar}$ (10% less than solid iron); $\beta = 70 \times 10^{-6} \text{ K}^{-1}$, $C_p = 10.2 \text{ cal mol K}$; and the relaxation time, $\tau =$

$5 \times 10^{-12} \text{ s}$. This relaxation time is typical of liquid metals⁹ (the other values are from refs 9, 11, 12, 13). $\Delta V/V$ is calculated from Fürth's relation. These parameters give

$$\eta_v = 3 \text{ cP}$$

and

$$\eta_s = (5/3)\eta_v = 5 \text{ cP}$$

for molten iron at low pressure. The measured value for η_s is 4.8–7.0 cP (ref. 14) and the near equality of η_s and η_v is consistent with measurements on other liquid metals⁹. Thus, the two-state theory gives satisfactory results at low pressure which leaves us to investigate the properties at core conditions.

The main effect of pressure and temperature is on the bulk modulus K and the relaxation time τ . The bulk modulus of the core is 4–10 times larger than iron at normal conditions^{11,15} and relaxation times have been estimated to be of the order of 10^{-8} – 10^{-9} s (ref. 16). Therefore, bulk viscosities of 4–6 orders of magnitude larger than at low pressures, or η_v of 10^2 – 10^4 P , are expected. The associated shear viscosities would be in the range of 10–100 P. The neglect of the effect of temperature and pressure on the other parameters would decrease these estimates slightly but probably by less than an order of magnitude. This estimate of η_s satisfies the lower limit (10 P) of Bukowski and Knopoff¹⁶ and the upper limit for the top of the outer core of 10^6 P based on the lack of damping of the 18.6-yr principal nutation¹⁷.

Effect of alloying

Based on density and cosmic abundances, the core is likely to contain up to 10% Ni and other siderophiles and a similar amount of light element such as O or S. There are additional volume relaxation mechanisms associated with alloys and solutions. In particular the relaxation times can be much longer than those due to shear and structural relaxations. This might be significant in explaining the apparent frequency dependence of Q of P waves in the outer core^{6,7}. There is a suggestion of a linear increase of Q_p with frequency which, if verified, indicates that the seismic band is on the high frequency side of the absorption band in the outer core. As this cannot be true for shear waves unless the unrelaxed shear modulus of the core is much lower than the rigidity of the inner core we may need a low-frequency volume relaxation mechanism. This contribution to the bulk attenuation and volume viscosity depends on concentration and may eventually shed light on the nature of alloying elements in the core.

Flinn *et al.*²¹ found a highly temperature-dependent absorption mechanism operating in binary melts which is not present in the pure metals.

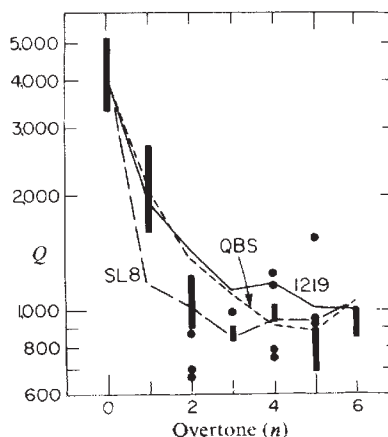


Fig. 1 Q values for the radial modes, ${}_nS_0$. The models SL8 and 1219 have moderate bulk attenuation in the core. Model QBS has high bulk attenuation in the upper mantle. The data are from ref. 2 and Table 1.

The extra absorption in liquid metal alloys is probably due to concentration fluctuations. Theoretically it is inversely proportional to the diffusivity or directly proportional to the shear viscosity. As with the structural viscosity it is also proportional to the square of the bulk modulus and can therefore, be expected to increase with pressure. More importantly it is a function of the curvature of the Gibbs potential versus concentration relation and, therefore, becomes very large in the vicinity of a critical point. Unfortunately, it is not possible to estimate the relaxation time of this mechanism in the core.

We conclude that structural and concentration fluctuations may be responsible for bulk viscosity in the core and contribute to the excess absorption of the radial modes.

Relaxation theory of bulk attenuation

The Q due to bulk losses in a relaxing solid or viscoelastic fluid is

$$Q_K^{-1}(\omega) = (\Delta K/K)\omega\tau_v/(1 + \omega^2\tau_v^2) \quad (2)$$

where $\Delta K/K$ is the modulus defect and τ_v is the volume relaxation time.

$\Delta K/K$ is the fractional difference between the high frequency and low frequency moduli. This has not been measured for liquid metals but is typically ~ 0.3 for other liquids. The decrease in bulk modulus on melting of metals ranges from ~ 5 to 30% . This may be of the same order as the modulus defect for molten metals. The bulk moduli of glasses are often taken as approximately the same as the high frequency moduli of fluids. This gives a modulus defect of 0.2 – 0.7 for most liquid–glass systems. These estimates give $Q_{\min}$ between 10 and ~ 3 at $\omega\tau = 1$. The increase in bulk modulus at the inner core–outer core boundary is about 5% (ref. 15). If this is taken as $\Delta K/K$ we obtain an estimate of 40 for the minimum Q_K of the core at $\omega\tau = 1$. A Q_K of 2×10^3 at a period of 20 min (the period of ${}_0S_0$) implies, from equation (2) that $\tau \sim 4$ s or 10^4 s. The smaller value would be consistent with the solid-like behaviour of the inner core for short-period body waves. The location of the inner core–outer core boundary would then be frequency dependent and the inner core would apparently be smaller at free oscillation periods. Such a frequency dependence has, in fact, been suggested by Gutenberg⁸. A frequency-dependent inner core boundary could explain the discrepancy between free oscillation and body wave determinations of the properties of the inner core. For example, the shear velocity obtained from PKJKP¹⁸ is $\sim 20\%$ smaller than that obtained from the free oscillation data^{15,19} for a fixed inner core radius. These results can equally well be interpreted in terms of a 20% decrease in inner core radius in going from body wave to free oscillation periods. Glass-like transitions, or transitions from fluid-like to solid-like behaviour typically take place over a small range of temperature. The sharpness of the inner core boundary could be explained by such a transition occurring over a small range of pressure.

Interpreting the data in this way requires that the seismic data

for the outer core be on the low frequency side of the relaxation peak. For the outer core

$$Q_K^{-1} = \frac{\Delta K}{K} (\omega\tau_v) \quad (3)$$

and, for the inner core

$$Q_K^{-1} = (\Delta K/K)/\omega\tau_v \quad (4)$$

The boundary of the inner core, then, corresponds to $\omega\tau = 1$ and this is where attenuation would be a maximum. For a 1 -s body wave the inferred relaxation time at the inner core–outer core boundary is $(2\pi f)^{-1}$ or ~ 0.2 s. This is close to the value deduced from Q_K in the outer core.

Bulk attenuation in solids

There are also several mechanisms that can contribute to bulk modulus or volume attenuation in solids. These include thermoelastic, magnetoelastic and phase changes. Because of the wavelength of seismic waves, thermoelastic losses would be due to heat flow between adjacent grains rather than from the compressional part to the dilatational part of the wave. Composites of anisotropic grains or grains of different compressibility should exhibit intergranular thermoelasticity²⁰. This depends on grain size, d , and thermal diffusivity, D . This mechanism gives a modulus defect and relaxation time of

$$\Delta M/M = \gamma\beta TR$$

$$\tau = d^2/3/\pi^2 D$$

where R is a measure of the grain anisotropy and varies between 0 and 1 .

For $\beta = 10^{-5} \text{ K}^{-1}$, $\gamma = 1$, $T = 2,000 \text{ K}$, $d = 1 \text{ cm}$ and $D = 0.001 \text{ cm}^2 \text{ s}^{-1}$, values appropriate for the mantle,

$$\Delta M/M = 0.02R$$

or

$$Q_{\min} = 100/R$$

and

$$\tau = 3 \text{ s}$$

The thermal diffusivity of iron at core conditions is $\sim 0.06 \text{ cm}^2 \text{ s}^{-1}$ (ref. 21) which shifts the characteristic thermal relaxation time to about 0.5 s for the inner core. Therefore, the intergranular thermoelastic mechanism will contribute most at body wave frequencies and for $R = 0.1$ gives a Q_K at ~ 1 s of the order of 10^3 . At a period of 20 min the Q_K associated with this mechanism will be $\sim 10^6$. A distribution of grain sizes will spread out the band and reduce the peak absorption.

For grain sizes of 1 cm or smaller the intergranular thermoelastic mechanism is small at free oscillation periods. Its presence, however, makes our estimate of Q_K in the core a lower bound. The low Q_K of the inner core at body wave frequencies may be due to this mechanism or due to the proximity to the relaxation peak in a viscoelastic fluid, as discussed earlier.

Conclusion

The damping of the radial modes can be satisfied by invoking a bulk dissipation in the core equivalent to Q_K (core) of 2×10^3 . This implies a bulk viscosity of 500 P . A two-state theory for bulk viscosity of liquid iron gives satisfactory results at low pressure and predicts a value at core pressures which is consistent with the radial mode data. The main uncertainty in the theoretical result is in the relaxation time. The same theory predicts a shear viscosity in the core which is several orders of magnitude lower. A relaxation theory of bulk viscosity suggests a relaxation time of the order of 1 s in the core. The same result is obtained if we assume that the inner core has relaxation times greater than body wave periods so that it seems to be a viscous fluid exhibiting unrelaxed moduli. Current estimates of the shear relaxation time in the core are highly uncertain, but are $\ll 1$ s. As structural relaxation times are expected to be the same

Table 2 Effect of bulk dissipation (Q_K) in mantle and core on Q of radial modes

	SL8*	1219	0220†	0279
Q_K (mantle)	∞	10^4	10^4	2×10^5
Q_K (core)	10^6	8×10^4	8×10^6	7×10^3
		4×10^5		
${}_0S_0$	4,374	4,310	4,803	4,062
${}_1S_0$	1,180	1,939	1,797	1,902
${}_2S_0$	1,024	1,459	1,351	1,381
${}_3S_0$	851	1,155	1,090	1,222
${}_4S_0$	945	1,206	1,072	1,136
${}_5S_0$	947	1,031	863	947
${}_6S_0$	1,057	1,015	834	873

* Ref. 3. Q_K (inner core) = 426–847.

† Q_K of outer core varies with depth.

as shear relaxation times this may suggest that a lower frequency mechanism, such as concentration fluctuations in an alloy, is responsible for the bulk viscosity.

If appreciable bulk attenuation occurs in the mantle and inner core, for example, by intergranular thermal currents, then the estimate of Q_K^{-1} in the outer core will be reduced and the estimate of the bulk viscosity will be an upper bound. The thermal relaxation time is not a strong function of temperature or pressure. It would, therefore, be a coincidence if it happened

to be near the period of $0S_0$. The viscous relaxation times, on the other hand, are strong functions of the external variables and it is not unreasonable that they sweep through the seismic band in the core.

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Isolation of the chicken thymidine kinase gene by plasmid rescue

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We have used the bacterial plasmid pBR322 as a vehicle to isolate genes coding for selectable markers from higher eukaryotes. In this way, we have obtained the chicken thymidine kinase (tk) gene as a 2.2-kilobase EcoRI/HindIII insert in pBR322. The cloned gene transforms tk⁻ animal cells with an efficiency equal to that of the cloned herpes simplex virus-1 tk gene.

SEVERAL genes of higher eukaryotes which encode major products of at least one tissue have been isolated, chiefly by using specific hybridization probes to screen genomic DNA fragments cloned into prokaryotic vectors¹⁻³. Recently, an efficient method has been developed for the stable genetic transformation of cultured animal cells⁴⁻⁷. Genomic DNA from wild-type cells has been used as donor to transfer genes coding for selectable markers, such as thymidine kinase, adenine phosphoribosyl transferase and hypoxanthine phosphoribosyl transferase, to mutant recipient cells⁸⁻¹¹. These developments make possible the isolation of genes coding for selectable markers. We describe here the isolation of a chicken thymidine kinase (tk) gene by means of 'plasmid rescue'. The chicken tk gene was chosen because an excellent recipient for DNA-mediated gene transfer was available in Ltk⁻, a tk-deficient mouse cell line, which has never been known to revert spontaneously to the tk⁺ phenotype⁸⁻¹². In addition, this gene has the advantage that both the tk⁺ and the tk⁻ phenotypes can be selected¹³.

Experimental design

In our scheme, chicken DNA is first digested with a restriction endonuclease which does not cleave tk and then ligated to similarly digested *Escherichia coli* plasmid pBR322 coding for ampicillin resistance (amp)¹⁴. This concatenated DNA is used to transform mouse Ltk⁻ cells to tk⁺. Some transformants can be expected to contain the chicken tk gene linked to pBR322 sequences. Next, the pBR322 sequences residing in the transformant are used to 'rescue' the flanking animal host sequences containing the tk gene. For this, DNA from the transformant is cleaved with a second restriction endonuclease, ligated in cyclization conditions and then used to transform *E. coli* to

ampicillin resistance. Plasmid clones resulting from transformation of *E. coli* are then tested for the ability to transfer tk back into tk⁻ animal cells. This scheme is illustrated in Fig. 1, in which the restriction endonuclease *Hind*III is used for the first set of cleavage/ligations and *Eco*RI is used for plasmid rescue.

Transformation of Ltk⁻ cells

Restriction endonuclease-cleaved chicken DNA was tested for the ability to transfer tk to Ltk⁻ recipients to identify the enzymes that did not cut the chicken tk gene. Among these were *Hind*III, *Bam*HI and *Eco*RI. *Hind*III was chosen for initial constructions. *Hind*III-cleaved chicken DNA was ligated to *Hind*III-cleaved pBR322 at 1:1 mass ratios, and then used to transform Ltk⁻ cells by the calcium phosphate co-precipitation method^{9,15}. We found that pBR322 sequences inhibited transformation in amounts exceeding 2 µg per 10⁶ cells. Therefore, 1 µg of chicken DNA which was ligated to 1 µg of pBR322 was co-precipitated in calcium phosphate along with 18 µg of Ltk⁻ DNA. This mixture was added to each plate containing 10⁶ cells. Cells were then selected in hypoxanthine/aminopterin/thymidine medium as reported before⁷. After 2 weeks, 12 tk⁺ colonies were cloned and cultured for further analysis.

All tk⁺ transformants were examined for the presence of pBR322 sequences by blot analysis^{16,17} using ³²P-labelled nick-translated^{18,19} plasmid as probe. Although 8 of 12 transformants contained 2-20 copies of pBR322, we could not assume that any of these was adjacent to the tk gene because far more pBR322 sequences than tk genes were present during transformation, and co-transformation with unlinked DNA fragments does occur¹⁷.

To establish the relationship between pBR322 sequences and the chicken tk gene, a second round of transformation and

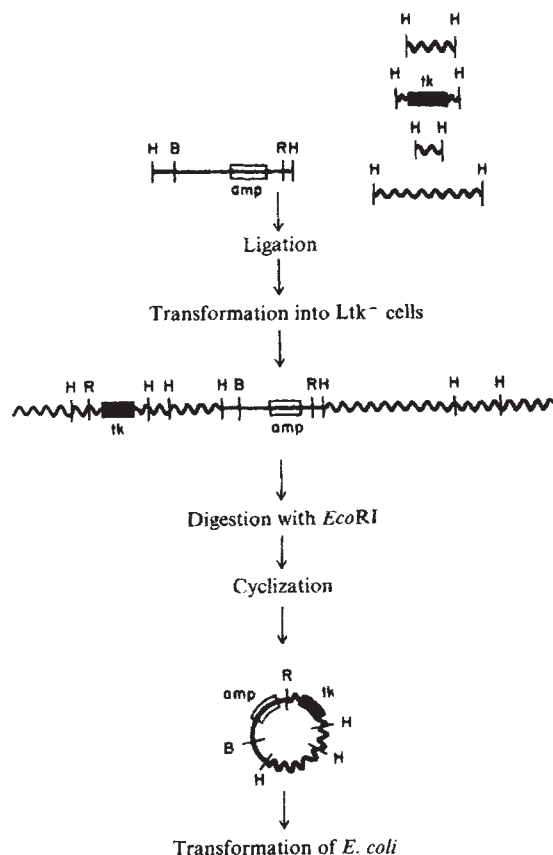


Fig. 1 A schematic illustration of the isolation of the chicken thymidine kinase gene (tk) by plasmid rescue. H, R and B refer to restriction endonuclease sites for *Hind*III, *Eco*RI and *Bam*HI, respectively (see text for details).

selection was necessary. Total DNA from each of four primary transformants containing pBR322 sequences was used to create eight secondary tk⁺ transformants. Blot analysis showed that 4 of the resulting 32 transformants (derived from 3 primary transformants) contained a single pBR322 sequence. Such sequences were not present in vast excess in the genomic DNA of primary transformants and so we concluded that the pBR322 sequences present in the secondary transformants were adjacent to the tk gene.

Rescue of pBR322 sequences

To rescue pBR322 sequences from transformed animal cells, these sequences must contain plasmid replication functions and an antibiotic resistance gene. Rtl-1a, a secondary transformant derived from the primary transformant tll, contained a *Hind*III fragment which annealed with nick-translated pBR322 and co-migrated with *Hind*III-cleaved pBR322. We concluded that no loss of pBR322 sequences occurred during transformation of this cell line. *Eco*RI and *Bam*HI were each used to rescue pBR322 from this line because they do not cleave the chicken tk gene and each cleaves pBR322 only once, on either side of the single *Hind*III site of the circular pBR322 molecule²⁰.

DNA from Rtl-1a was digested, ligated in cyclization conditions, and used to transform *E. coli* χ 1776 (ref. 21) by a high efficiency procedure (D.H., in preparation). The efficiency of transformation of χ 1776 with this cellular DNA was about 100-fold lower than expected from reconstruction experiments. Two ampicillin-resistant colonies were obtained which contained pBR322 derivatives: a 9.2-kilobase plasmid, pR-1, derived from *Eco*RI cleavage, and an 8.5-kilobase plasmid, pB-1, derived from *Bam*HI cleavage. Restriction maps of these plasmids are presented in Fig. 2.

We next verified (by hybridization) that the rescued plasmids were derived from the transformed mouse cells. Rescued

sequences were nick translated and used as probes in blot analysis of restriction endonuclease-cleaved Rtl-1a DNA. Figure 3a and b show that *Bam*HI-cleaved pB-1 co-migrated with an homologous *Bam*HI fragment of Rtl-1a. Furthermore, Fig. 3c and d show that *Hind*III/*Bam*HI-cleaved pB-1 co-migrated with homologous fragments in *Hind*III/*Bam*HI-cleaved Rtl-1a. Double digestion with *Hind*III and *Bam*HI yielded two fragments not resolved in these conditions. Similarly, Fig. 4a-d show that *Eco*RI-cleaved pR-1 co-migrated with an homologous *Eco*RI fragment of Rtl-1a and that *Eco*RI/*Hind*III cleavage products of pR-1 each co-migrated with homologous fragments present in *Eco*RI/*Hind*III-cleaved Rtl-1a. The 0.6-kilobase *Hind*III/*Hind*III fragment is not visible.

We conclude that both pR-1 and pB-1 were rescued from animal cells into *E. coli*. From their respective restriction maps, a restriction map was reconstructed of pBR322 and its flanking host sequences in Rtl-1a (Fig. 2). This map was verified independently by blot analysis after cleavage of Rtl-1a DNA with a variety of restriction endonucleases.

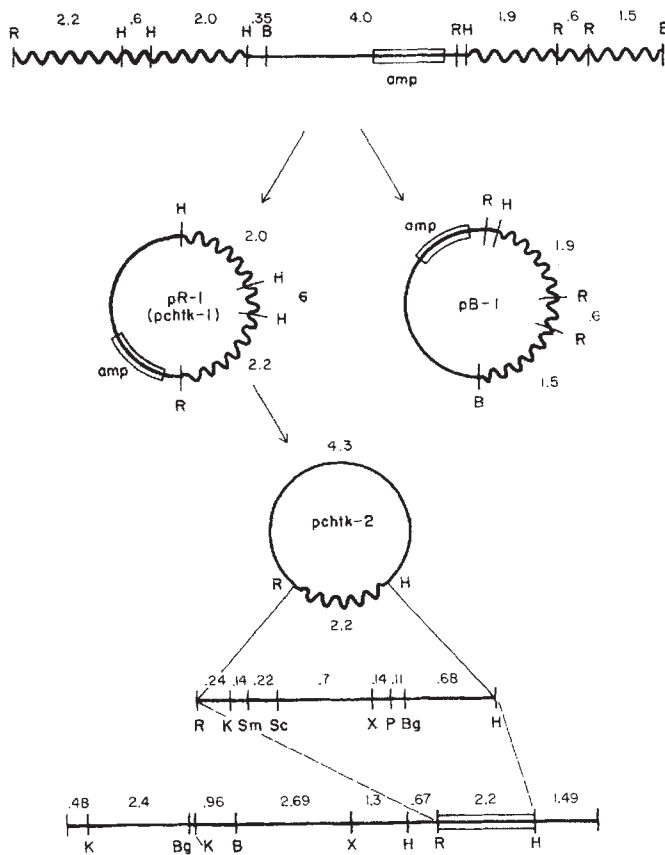


Fig. 2 Restriction maps and derivations of rescued plasmids. DNA from the secondary transformant Rtl-1a was prepared as previously described¹⁷ and cleaved with either *Eco*RI or *Bam*HI. Digested DNA was then cyclized with T4 DNA ligase at a DNA concentration of 5 μ g ml⁻¹. Cyclized DNA was used to transform *E. coli* χ 1776 by a procedure to be described elsewhere (D.H., in preparation). Two plasmids, pR-1 and pB-1, were obtained from *Eco*RI-cleaved and *Bam*HI-cleaved, cyclized DNA, respectively. Restriction maps of these plasmids are shown, with pBR322 sequences denoted "—". The numbers refer to kilobase pairs. From the maps of the two rescuants, a restriction map was reconstructed of the sequences flanking the pBR322 sequence resident in Rtl-1a (top line). pR-1 was found to encode tk and was renamed pcht-1. pcht-1 was cleaved with *Hind*III and cyclized to construct pcht-2, which contains only the 2.2-kilobase *Eco*RI/*Hind*III insert encoding tk. Below is a restriction map (to scale) of the 2.2-kilobase insert. At the bottom is a restriction map (to scale) of the 12.5-kilobase insert of pcht-1 isolated from a Charon 4A chicken library²². *Pvu*II, *Sma*I and *Sac*I sites are not mapped on the phage. Restriction enzymes are *Bam*HI (B), *Eco*RI (R), *Hind*III (H), *Kpn*I (K), *Sma*I (Sm), *Sac*I (Sc), *Xba*I (X), *Pvu*II (P) and *Bgl*II (Bg). Restriction enzyme digestions and ligations carried out in conditions described by the vendors (Bethesda Research Labs and New England Biolabs).

Table 1 Transformation data

Gene source	Restriction cleaved*	Total colonies†	Specific activity‡
pchtk-1	Uncut	1,628§	14.7
pchtk-2	Uncut	1,075§	7.6
pchtk-2	<i>EcoRI</i>	612§	4.3
pchtk-2	<i>KpnI</i>	0§	0.00
pchtk-2	<i>SmaI</i>	3§	0.02
pchtk-2	<i>SacI</i>	0§	0.00
pchtk-2	<i>XbaI</i>	0§	0.00
pchtk-2	<i>PvuII</i>	227§	1.6
pchtk-2	<i>BglII</i>	618§	4.4
pchtk-2	<i>HindIII</i>	1,863§	13.0
λchtk-1	Uncut	255	55.0
pTK-2 #	<i>SalI</i>	690	29.2
Chicken, genomic DNA	Uncut	51	63.7

* Three units of enzyme were used to digest 1 µg of DNA for 4 h at 37 °C in buffers recommended by the vendors.

† Transformations were carried out as previously described⁹. Ltk⁻ DNA was used as carrier for cloned genes at 20 µg per plate. For genomic chicken transformations, the DNA was its own carrier.

‡ Colonies per 10⁶ molecules per 10⁶ cells. The DNA content of chicken is taken as 2.5 pg per cell.

§ Total number of colonies in five plates, 20 ng gene per plate.

|| Total number of colonies in five plates, 4 ng gene per plate.

¶ Total number of colonies in five plates, 20 µg per plate.

The 3.5-kilobase *Bam*HI fragment of HSV-1 strain F (ref. 7) containing the HSV-1 tk gene was cloned into the *Bam*HI site of pBR322. The orientation of the tk fragment is the same as the pFG5 clone of Colbere-Garapin *et al.*²⁵.

The sequences flanking pBR322 in the secondary transformant Rtl-1a could theoretically have arisen from chicken or mouse (or both), depending on events occurring during transformation. Blot analysis showed that the sequences contained in both pR- and pB-1 were derived entirely from chicken. The *Hind*III/*Bam*HI insert of pB-1 co-migrated with an homologous *Hind*III/*Bam*HI fragment of chicken DNA (Fig. 3c, e) whereas pB-1 showed no homology to mouse DNA in stringent hybridization conditions (Fig. 3f-j). Similarly, *Hind*III/*Eco*RI digestion of pR-1 generated one fragment of 4.3 kilobases containing only pBR322 sequences and three fragments of 2.2, 2.0 and 0.6 kilobases. These latter three fragments co-migrated with homologous fragments in chicken DNA cleaved with *Hind*III and *Eco*RI (Fig. 4c, e). Moreover, pR-1 showed no homology to mouse DNA (Fig. 4j).

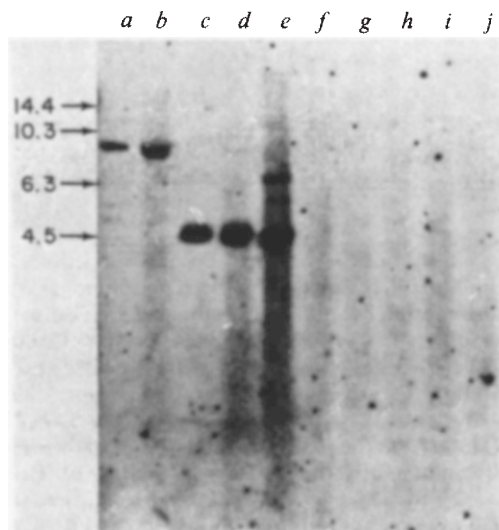


Fig. 3 Blot analysis of the pB-1 rescuant. pB-1 was ³²P labelled to high specific activity (2 × 10⁶ c.p.m. per µg) by nick translation¹⁹ and used as probe in Southern blots¹⁷ to DNA electrophoresed in 1.0% agarose gels: a, *Bam*HI-cleaved pB-1 (40 pg with 2 µg salmon DNA carrier); b, *Bam*HI-cleaved Rtl-1a (10 µg); c, *Bam*HI- and *Hind*III-cleaved DNA from: c, pB-1 (40 pg, with carrier); d, Rtl-1a (10 µg); e, chicken (10 µg); f, i, various secondary, chicken tk⁺ mouse transformants (primary transformant tll used as donor) (10 µg each); j, Ltk⁻ (10 µg). Arrows on the left are sizes (in kilobases) of restriction fragments of adenovirus-2. Digestions were in 30 µl final volume, 2 units of enzyme per µg DNA for 3 h at 37 °C.

pR-1 contains the chicken tk gene

pR-1 and pB-1 were tested in transformation assays for ability to transfer tk activity to Ltk⁻ cells. Although pB-1 gave no tk⁺ colonies, treatment of mouse cells with pR-1 yielded approximately 10 tk⁺ colonies per ng, only slightly below the frequency observed with a pBR322 clone containing the 3.5-kilobase *Bam*HI fragment of HSV-1 which encodes the herpes simplex virus tk gene (pTK-2) (Table 1). These observations strongly suggested that pR-1 encoded the chicken tk gene, and this was confirmed by hybridization studies. As expected, pB-1 showed no homology to chicken tk⁺ mouse transformants (Fig. 3). On the other hand, pR-1 showed homology to chicken tk⁺ mouse lines but not to Ltk⁻ itself (Fig. 4f-j). Moreover, mouse cells transformed with pR-1 contained these chicken DNA sequences (Fig. 5b, c, d). No transformants contained HSV-1 tk sequences. Further confirmation was obtained by comparing the physical properties of the tk activity expressed in transformants with that of chicken, mouse and herpes tk. Isoelectric focusing of cytoplasmic extracts showed that transformants obtained using pR-1 as gene donor expressed a tk activity with the same isoelectric point (9.7) as that of 6-day-old chick embryo extracts and distinguishable from mouse (9.0) and herpes (6.1)²². We therefore conclude that pR-1 encoded the chicken tk gene. pR-1 was renamed pchtk-1.

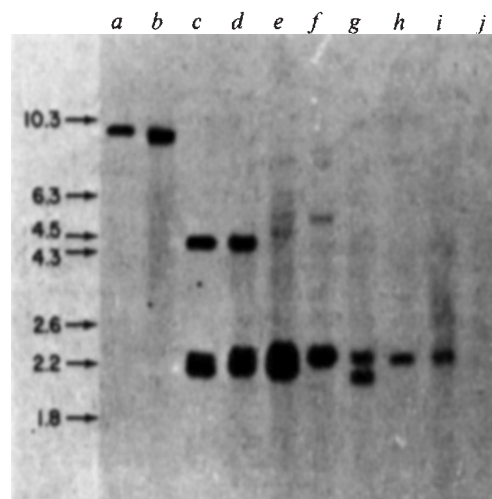


Fig. 4 Blot analysis of the pR-1 (pchtk-1) rescuant. pR-1 was used as probe in blots to DNA electrophoresed in 1.0% agarose gels: a, *Eco*RI-cleaved pR-1 (40 pg with 2 µg salmon carrier); b, *Eco*RI-cleaved Rtl-1a DNA (10 µg); c, *Eco*RI- and *Hind*III-cleaved DNA from: c, pR-1 (40 pg with carrier); d, Rtl-1a (10 µg); e, chicken (10 µg); f, g, h, various secondary chicken tk⁺ mouse transformants (primary transformant tll used as donor) (10 µg); i, a chicken tk⁺ primary transformant (10 µg); j, Ltk⁻ (10 µg). Arrows indicate sizes (in kilobases) of restriction fragments of adenovirus-2. Digestions were as in Fig. 3.

Localizing the chicken tk gene

pchtk-1 contains three chicken fragments bounded by *Eco*RI and/or *Hind*III sites: a 2.2-kilobase *Hind*III/*Eco*RI fragment, a 2.0-kilobase *Hind*III/*Hind*III fragment and a 0.6-kilobase *Hind*III/*Hind*III fragment. From its construction, we expected that the three chicken specific fragments of pchtk-1 were not contiguous in the chicken genome (Fig. 1). Thus, any of these fragments could have encoded the chicken tk gene. Blot analysis using nick-translated pchtk-1 as a probe showed that DNA from chicken and chicken tk⁺ mouse transformants contained in common only a 3.0-kilobase *Hind*III fragment. Thus, the 2.2-kilobase *Eco*RI/*Hind*III fragment contained the active gene. To test this rigorously, a second plasmid, pchtk-2, was derived from pchtk-1 by cleaving it with *Hind*III, cyclizing it and using it to transform *E. coli*. The resultant plasmid contained only the 2.2-kilobase *Hind*III/*Eco*RI fragment inserted into pBR322. This plasmid transformed Ltk⁻ to the tk⁺ phenotype with an

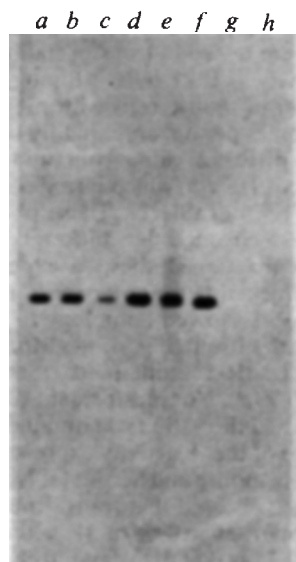


Fig. 5 Presence of the 1.4-kilobase *EcoRI/PvuII* fragment in transformants, chicken, plasmid and phage DNA. DNAs from various sources were digested with *EcoRI/PvuII*, electrophoresed in 1.5% agarose gels, transferred to nitrocellulose filters and hybridized *in situ* with ^{32}P -labelled, nick-translated, *KpnI/XbaI* fragment of chicken tk (2×10^8 c.p.m. per μg). a, Charon 4A clone, $\lambda\text{ctk-1}$ (200 pg); b–d, three different tk $^{-}$ mouse transformants obtained using pchk-1 as gene donor (8 μg each); e, chicken (8 μg); f, pchk-2 (50 pg with 2 μg salmon DNA carrier); g, HSV-1 tk $^{+}$ mouse transformant (8 μg); h, Ltk $^{-}$ (8 μg).

efficiency approximately equal to that of pchk-1 (Table 1).

To localize the tk gene in more detail, we identified restriction sites on the 2.2-kilobase *HindIII/EcoRI* fragment using enzymes that cleave the fragment once (Fig. 2). pchk-2 was cleaved to completion with each enzyme and the linearized plasmid was used in transformation assays (Table 1). By this procedure, we localized one end of the tk gene to the fragment bounded by the *EcoRI* and *KpnI* sites, and the other end to the fragment bounded by the *XbaI* and *PvuII* sites. Thus, a functional chicken tk gene is no larger than 1.4 kilobases and may be as small as 1.0 kilobase.

The plasmid carrying the biologically active chicken tk gene has had a complex history: it was passed through mouse cells as well as through *E. coli*. To verify the structure of the tk gene independently, we have cloned it by an alternative and more

familiar route. A library of chicken DNA^{23,24} distributed into the bacteriophage λ vector, Charon 4A, was screened using nick-translated pchk-2 as probe. DNA from one cloned phage ($\lambda\text{ctk-1}$) contained a 12.5-kilobase chicken insert which transferred tk efficiently to Ltk $^{-}$ cells (Table 1). Restriction mapping and blot analysis (Figs 2, 5) confirmed the colinearity of the tk genes cloned in phage and by plasmid rescue.

The scheme we have demonstrated for the isolation of the chicken tk gene represents the first instance of the purification of a higher eukaryotic gene using essentially genetic techniques. In principle, this method is applicable to any higher eukaryotic gene coding for a selectable marker. We emphasize, however, that the isolation of tk has been greatly facilitated by its small size, a strong selection system and an excellent recipient cell used to detect its transfer.

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LETTERS

An upper limit on the EUV flux from HD192273

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Cash *et al.*¹ reported the possible detection of an extreme ultraviolet (EUV) source in the constellation Pavo. They suggested HD192273 as a possible candidate and cited a TD-1 satellite observation indicating that this star is also unexpectedly bright at 1,565 Å. Wegner² reported visual photometric and spectroscopic data for HD192273 and concluded that it is

apparently a normal B-type star. Shipman and Wegner³ have reviewed the available data on HD192273 and suggested that all of the observations could be explained if this object were an analogue of HZ22, an evolved, mass-exchange, binary. We report here a recent observation with the UV spectrometer (UVS) aboard the Voyager 1 spacecraft. An upper limit (2σ) of 8×10^{-3} photon $\text{cm}^{-2} \text{s}^{-1} \text{Å}^{-1}$ is placed on the 534–776 Å flux from HD192273 at the time of observation. This is $\sim 10\%$ of the 500–780 Å flux reported from Pavo by Cash *et al.* The 1,565 Å flux observed by TD-1 is confirmed and is consistent with the stellar parameters determined by Wegner. Discounting the possibility that HD192273 is an EUV variable, it is probably not the potential source reported by Cash *et al.*

The Voyager 1 UVS is an objective grating instrument covering 534–1,701 Å with 126 contiguous photon counting pixels having maximum sensitivity shortward of 1,000 Å (refs 4, 5). Pre-flight laboratory calibrations were based on calibrated channeltron detectors, traceable to NBS standard photodiodes, and indicate a transfer error of $\pm 15\%$ from the photodiodes. The in-flight EUV sensitivity has been demonstrated in several

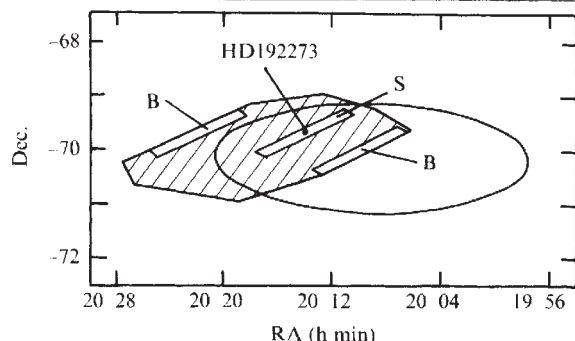


Fig. 1 The shaded area shows the portion of the sky mapped in this observation. The $0.1 \times 0.86^\circ$ UVS field of view is shown in three positions corresponding to the spatial bins from which the stellar signal (S) and the background spectra (B) were taken. The ellipse shows the position of the 100% response contour of Cash *et al.* when a possible detection of an EUV source was made. Further mapping of this area by the Voyager UVS is planned.

ways: the Voyager 1 UVS has observed the integrated solar disk. Comparison with the published solar spectrum⁶ confirms the EUV sensitivity calibration. UVS measurements of Io's ion torus⁷ (534–1,000 Å) are consistent with the optical observations at other wavelengths, *in situ* measurements, and theoretical models of the torus. The recorded strength of He I 584 Å scattered by the interstellar medium confirms the EUV sensitivity of the UVS during the observation reported here.

The UVS was pointed towards HD192273 between 01.14 UT on 27 November 1979 and 21.13 UT on 28 November 1979. Spacecraft attitude control motions caused the field of view to wander over a portion of the sky shown in Fig. 1. Using pointing information, the spectra were accumulated into spatial bins. The bin (S in Fig. 1) having the strongest signal in the 912–1,701 Å range corresponds in position to HD192273. No other source was detected within the shaded area. A background spectrum was accumulated from the two areas B on Fig. 1. This background spectrum (total integration time 2,496s) was appropriately subtracted from the stellar spectrum (total integration time 2,880s) to correct for the He I 584 Å and H Ly α emission scattered by the interstellar medium plus any cosmic UV radiation.

No statistically significant signal was found in the 534–776 Å range. The measured count rate corresponds to a flux of $(2 \pm 3) \times 10^{-3}$ photon $\text{cm}^{-2} \text{s}^{-1} \text{Å}^{-1}$ over the wavelength range. The stated 1σ uncertainty includes counting statistics and the background subtraction. The upper limit (2σ) on the EUV flux is 8×10^{-3} photon $\text{cm}^{-2} \text{s}^{-1} \text{Å}^{-1}$ or about 10% of the flux reported by Cash *et al.* from Pavo. Of course, not all the area included in their field has been mapped in this observation, and it is possible that a source exists outside the area over which the observations overlap. Nor can we exclude the possibility that the EUV source is variable. However, it is unlikely that HD192273 varies by more than a factor of 10 in the EUV as it is not known to vary in the visible or UV. The UVS observed a 1,396–1,701 Å flux of 1.4 ± 0.3 photon $\text{cm}^{-2} \text{s}^{-1} \text{Å}^{-1}$ in fair agreement with the flux of 1.7 photon $\text{cm}^{-2} \text{s}^{-1} \text{Å}^{-1}$ observed by TD-1 in the 1,400–1,730 Å band⁸.

The latter (1,565 Å) flux is too bright for the Henry Draper type of B8 but not for an earlier spectral type. Wegner determined $T_e = 27,000$ K and $\log g = 4.8$ from his visual observations. Using the model atmosphere results of Kurucz⁹ for these parameters, the theoretical ratio of the flux in the 1,565 Å band to the flux in the V band was estimated to be ~ 30 . The absolute calibration of α LYR (ref. 10) provides a relation between apparent magnitude and flux. The observed² $V = 8.83$ for HD192273 and the magnitude-flux relation imply a predicted flux of 1.2 photon $\text{cm}^{-2} \text{s}^{-1} \text{Å}^{-1}$ at 1,565 Å which is in reasonable agreement with both the observed Voyager 1 and TD-1 fluxes.

We conclude that HD192273 is probably not the 'potential' EUV source reported by Cash *et al.* and that it is not abnormally bright at 1,565 Å for its effective temperature and gravity. However, the problem of understanding its apparently large distance from the galactic plane in terms of its small radial velocity and short life expectancy remains.

Note added in proof: Additional UVS observations of the Pavo region were obtained in February 1980. Observations of HD192273 on 6 and 18 February are consistent with the upper limit on EUV flux given above. A search of 95% of the field of Cash *et al.*¹ showed no evidence of an EUV source; a 2σ upper limit of 3.2×10^{-2} photon $\text{cm}^{-2} \text{s}^{-1} \text{Å}^{-1}$ can be placed on any source within the area. In addition, no FUV (911–1,200 Å) sources other than HD192273 were found within the search area, down to a sensitivity threshold of 0.08 the brightness of HD192273. It is unlikely that HD192273 is an EUV variable, as no EUV flux has been detected in three separate observations.

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Spectra of SO₂ frost for application to emission observations of Io

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We have obtained, and describe here, laboratory reflection spectra of SO₂ frost from 6 to 14 μm . For rapid deposition of the frost layer at a deposition temperature of 80 K, the frost exhibits a strong absorption band at 7.5 μm , consistent with the possible Voyager I identification of such a band due to suspended solid SO₂ particles within Io's atmosphere. This compatibility does not, however, exist if the frost layer is formed at 140 K or if the layer is formed slowly. Thus it may be possible to obtain information concerning the particle formation process using more detailed laboratory SO₂ frost spectra.

Although spectra of the Galilean satellites Europa, Ganymede and Callisto clearly indicate water ice absorption features representing free water in the form of surface frost¹, no water ice features have been identified in reflection spectra of Io. Instead, it has been suggested^{2,3} that observed Io absorption bands in the near IR represent solid SO₂. The presence of solid SO₂ on Io's surface is compatible with the recent Voyager I IR experiment⁴, from which gaseous SO₂ was identified on Io, with an estimated abundance consistent with a gaseous SO₂ atmosphere which is in equilibrium with solid SO₂ at the local surface temperature. As Pearl *et al.*⁴ have pointed out, an obvious source of gaseous SO₂ consists of volcanic plumes with possible precipitation of solid

SO₂ particles from the plumes. This hypothesis would be compatible with the suggestive identification of solid SO₂ within Io's atmosphere from Voyager I emission observations over a warm region containing a volcanic feature⁴. This tentative identification refers to a possible weak absorption between 1,320 and 1,340 cm⁻¹ (7.5 μm). Existing laboratory transmission spectra⁵⁻⁸, for formation temperatures ranging from 20 to 93 K place the ν₃ band of solid SO₂ in this region.

But the spectra of condensed phases depends on several factors. For example, Slobodkin, *et al.*⁹ have shown that the spectral locations of solid NH₃ bands can depend on deposition rate and deposition temperature. Moreover, they emphasize that solid particles, comprising either a cloud or a haze, are formed rapidly, and that laboratory frost-layer simulation should attempt to mimic such a rapid formation process. The point is that the polycrystalline structure of the solid is influenced by formation rate. In view of the considerable current interest concerning the composition of Io's surface and atmosphere, we have obtained new IR SO₂ frost spectra for a limited range of deposition rates and deposition temperatures. Reflection spectra have been obtained from 6 to 14 μm for 3-mm thick frost layers which were formed at 80 K, subsequently warmed to 140 K, and then cooled back to 80 K. Because, for a given formation rate, there is no distinction between warming a frost layer to a given temperature or forming it at that temperature⁹, the 140 K spectra would be representative of a 140 K formation temperature. Two deposition rates were used; slow deposition at 7.5 μm min⁻¹ and rapid deposition at 1,500 μm min⁻¹.

The slow deposition spectra are shown in Fig. 1. The rather minor changes in the spectra on warming from 80 to 140 K could be indicative of a polycrystalline phase transition. The 80 K (cooled from 140 K) spectrum is quite similar to the 140 K spectrum, as would be expected, as phase transitions do not occur on cooling. The 8.9-μm feature in this spectrum seems to be a blend of the 8.8-μm band and a weaker 9-μm feature, both of which appear in the other two spectra, rather than a shift in the location of the 8.8-μm band. The spectra in Fig. 1 do not agree with the transmission spectra for 20–93 K formation⁵⁻⁸. In these studies the ν₁ and ν₃ bands are located at 8.7 and 7.5 μm, respectively. Our spectra indicate a slightly shifted ν₁ band, and there are considerable differences in the 7–8 μm region. But this seems to be due to formation rate differences.

Our rapid deposition spectra are shown in Fig. 2. For 80 K formation the 7.5 and 8.7 μm band locations coincide precisely with the 20–93 K transmission spectra⁵⁻⁸. This suggests that the polycrystalline phase which was produced in obtaining the

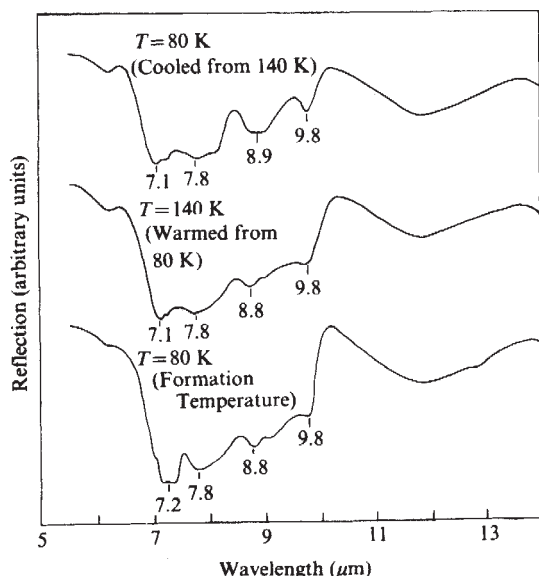


Fig. 1 Solid SO₂ reflection spectra for a frost layer formed by slow deposition (7.5 μm min⁻¹).

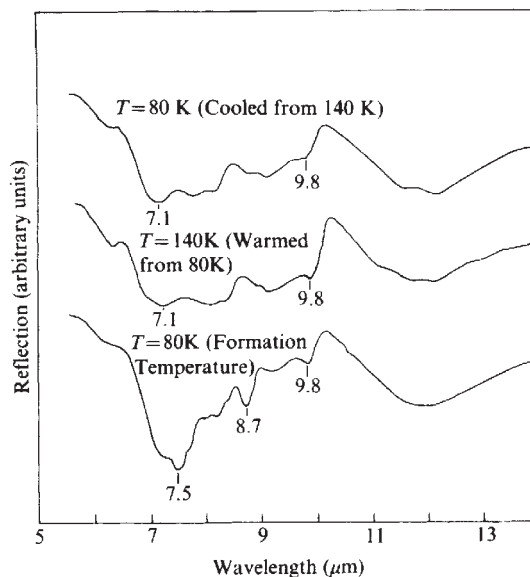


Fig. 2 Solid SO₂ reflection spectra for a frost layer formed by rapid deposition (1,500 μm min⁻¹).

transmission spectra actually coincides with our rapid-deposition frost layers. The 9.8-μm band, which appears clearly in all our spectra, is not mentioned in any of the transmission studies. But as pointed out by Herr and Pimentel¹⁰, reflection spectra, in contrast with transmission spectra, tend to enhance weak absorption features, and thus the possibility exists that the 9.8-μm band was simply not evident within transmission spectra. Indeed Smythe *et al.*², in their near-IR reflection study, have identified several solid SO₂ bands which apparently were not evident in the most detailed of the transmission spectra⁷. The weak band at 6.2 μm, as well as the broad feature at ~11.8 μm, are most likely due to contaminant water which is also present in the near-IR reflection spectra of Smythe *et al.*², and Fanale *et al.*³.

As previously discussed, we anticipate the rapid deposition frost layer to be representative of solid SO₂ particles formed within Io's atmosphere, and this is consistent with the possible identification of a solid SO₂ absorption feature at 7.5 μm from the Voyager I emission observations⁴. But this would only be compatible with the 80 K formation spectrum of Fig. 2. Evidently, at least for rapid deposition, there is a significant alteration in the structure of our frost layer on warming from 80 to 140 K, with the 140 K spectrum showing no feature which could be attributed to the possible Voyager I identification. This structural change could be partially due to changes in grain size on warming the layer, but grain-size changes should not induce a shift in band locations¹¹. Instead, at least for rapid deposition, a significant polycrystalline phase transition is probably the primary cause of the alteration in our rapid-deposition spectra on warming from 80 to 140 K. The consistency of our rapid-deposition 80 K spectrum with the 93 K transmission spectra^{5,6} would in turn imply that the phase transition occurs between 93 and 140 K. This further suggests that if the observed Voyager I absorption feature is due to solid SO₂ particles within Io's atmosphere, then the particles are formed at a temperature below 140 K.

If the above hypothesis is correct, it would be useful for diagnostic purposes to have laboratory spectra available covering a broad range of wavelengths and formation temperatures. We plan to obtain such spectra.

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A fluid-dynamical model of differentiation and layering in magma chambers

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Large igneous intrusions characteristically have a systematic gradation in mineral composition from top to bottom, often associated with a horizontally layered structure on several scales^{1,2}. Current theories³ invoke the settling of denser crystals as the mechanism responsible for separating the various components from the solidifying melt. However, recent laboratory experiments^{4,5}, using crystallization from aqueous solutions to model the essential fluid-dynamical processes, have documented another significant way in which chemical differentiation of the liquid can be caused by relative motion between crystals and the remaining melt. The growth of crystals of a denser component on a side-wall boundary leaves behind less dense fluid. (This corresponds to the behaviour of many common magmas, notably those of the calc-alkaline series. However, in tholeiitic basalt magmas, of the type which commonly form layered intrusions, crystallization leads to an increase in density of the remaining melt.) The lighter fluid rises to the top of the chamber in a boundary layer flow, which builds up a stable density gradient, by the 'filling box' mechanism^{6,7} previously discussed in other contexts. In the latest experiments reported here, the process of differentiation is demonstrated explicitly: starting with a homogeneous mixture of two solutes in water, crystallization at a vertical boundary produces overall vertical composition gradients, as well as density stratification accompanied by smaller scale layering.

Previous, more extensive experiments⁴ have systematically examined the additional effects due to crystallization in a 'double-diffusive' system* (that is, one in which the difference in molecular diffusivities of the several components has an important role). Sodium carbonate solution was chosen as the working fluid, because of its high solubility and large change in solubility with temperature, and various initial density distributions and cooling configurations were explored. Potassium carbonate has been used as the second solute in both the previous and the present experiments. The geometry adopted for the new experiments was suggested by the observations⁹ of basaltic pillars on the sea floor, which are cooled by water circulating through a hollow conduit along their centres, but this analogy is immaterial for the present purpose and will not be pursued further here. The results are believed to have a more general significance, and depend only on the occurrence of crystallization at any side boundary.

There has been much recent discussion¹⁰ about the non-newtonian behaviour of natural magmas. A finite yield strength

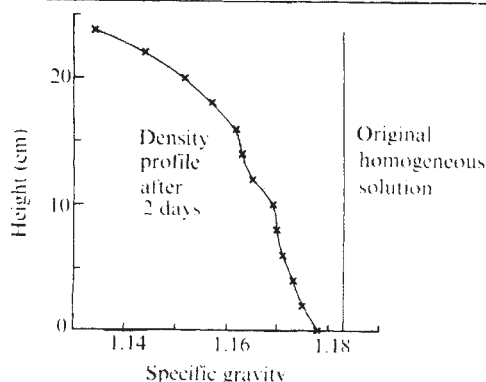


Fig. 1 The density profile produced in Na_2CO_3 solution, by the upflow accompanying crystallization at a central rod, 47.5h after cooling began. The initial homogeneous density distribution is also shown. (The refractive index of small samples withdrawn from the tank was the property actually measured, with a calibration to relate this to density.)

can certainly inhibit the settling of small crystals, but such effects should be less important in the present case. First, crystallization at a boundary will leave the adjacent melt relatively free of crystals in suspension, so removing one likely cause of a non-newtonian viscosity. Second, a boundary layer of less dense fluid with increasing total buoyancy will eventually begin to flow upwards, whatever the nature of the viscosity. Our experiments using aqueous solutions should thus reproduce the important overall features of the prototype flows.

The experimental tank was made of Perspex, 40 cm $\times$ 20 cm in cross-section and 30 cm deep. It was fitted with a 9-mm o.d. copper tube, projecting vertically through a seal in the centre of the base and extending the whole depth of the tank. All the runs described below were started by filling the tank to a depth of ~25 cm with a homogeneous solution (or mixture of solutions), covering it with a floating, insulating lid, and then cooling it from the centre by circulating liquid coolant through the central tube from a bath held at known temperature.

Consider first an experiment using a single solute, Na_2CO_3 in an aqueous solution of specific gravity 1.183. When coolant, at 0 °C, was pumped through the central tube, a cold downflow was observed near the central rod. After a few minutes, however, crystallization began, with the crystals adhering to the cooled surface, and a strong upflow was seen instead. This upflow occurs because the formation of crystals of $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$, of specific gravity 1.44, leaves behind a boundary layer of depleted solution which is less dense. This layer, in which the change of concentration more than compensates for the density increase due to the decreased temperature, rises to the top of the chamber. The lightest (most depleted) solution is continually fed



Fig. 2 Shadowgraph of the experiment described in the caption to Fig. 1. The axisymmetric crystal mass, shown in silhouette, is surrounded by 'double-diffusive' layers, produced by cooling the stable density gradient at the centre.



Fig. 3 Shadowgraph showing the layers in the fluid, and associated scallops on the crystals, formed by cooling a homogeneous solution of Na_2CO_3 . (Initial specific gravity=1.164, coolant temperature -2.5°C . Photograph at 44 h.)

to the top of the region, and density stratification is eventually built up (see Fig. 1).

Another striking feature of this stratification is shown in Fig. 2, taken just before the density profile was measured. The shadowgraph reveals a series of layers (of which there are also some indications in the rather coarse density profile of Fig. 1). These are a consequence of the double diffusive instabilities characteristic of top and side cooling of a stable composition gradient⁸. In the present case, the side cooling probably dominates, and the layering is equivalent to that produced near a melting iceblock; this effect has recently been studied^{11,12} in the context of melting icebergs.

The upflow in the boundary layer which feeds light fluid to the top of the box results in the individual layers being pushed downwards with time. Thus the crystals do not in general remain in the same relation to the interfaces as they grow, and any persistent layer structure in the crystals tends to be obliterated. At a later stage of other experiments, however, the interfaces did stay at a fixed level, and the growth (or in some cases resolution) of the sodium carbonate crystals is then clearly affected by the existence of, and circulation in, the fluid layers (see Fig. 3). Again there are close analogies to what has been observed¹² during the melting of vertical ice walls.

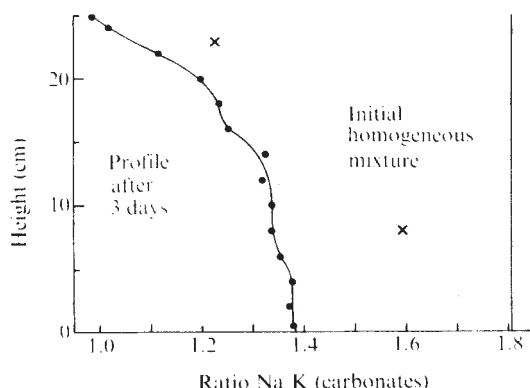


Fig. 4 Measurements of the ratio Na/K concentrations in solution as a function of depth, produced by crystallization of $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ from an initially homogeneous mixture of sodium and potassium carbonates. $\times$, Spot measurements after 1 day; $\bullet$, profile after 3 days (Na and K concentrations measured using atomic absorption spectrophotometry after appropriate dilution).

The behaviour to be expected when there is more than one solute can be deduced (at least in retrospect) from what has already been presented, but this case is of such importance that it is worth studying directly. The tank was filled with a homogeneous mixed solution of specific gravity 1.358, made up of Na_2CO_3 (21.45 wt %) and K_2CO_3 (11.87 wt %). In this case, the coolant was gradually brought down to 0°C from room temperature (20.4°C). Crystals nucleating near the central rod at first did not remain attached, but fell out to form a conical pile at the base. As the temperature dropped to about 12°C , the crystals attached themselves, the upflow of lighter fluid began, and the chamber stratified as described previously. It was found by later analysis of the crystals that pure $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ was again crystallizing out. Thus as well as being depleted in sodium, the upward flowing boundary layer was relatively enriched in potassium. Instead of recording the density distribution, it is now more instructive to plot the Na/K ratio (Fig. 4). This demonstrates directly that 'differentiation' has been produced in the experimental tank. There is a systematic variation in composition of the solution with depth which becomes more pronounced with time.

Layering is also observed in this case, as shown in Fig. 5. Several of the interfaces can be clearly identified with steps in the concentration profiles, and in the associated density profile (which was measured, but is not reproduced here). Note also the smaller steps and sharp interfaces near the top of the tank. We suspect that these are 'diffusive' interfaces produced by the



Fig. 5 Shadowgraph of the experiment described in the caption to Fig. 4, taken just before the samples were withdrawn for the concentration profile measurement. In addition to the features seen in Fig. 2, note the fine-scale 'diffusive' layers at the top.

opposing vertical composition gradients (K_2CO_3 being relatively more concentrated at the top), rather than by the horizontal temperature gradients responsible for the formation of the deeper layers. Similar structures have been observed in earlier experiments⁴.

In conclusion, we re-emphasize that the deductions made above do not depend critically on the particular geometry chosen, or on the complexity of the crystallizing system. The scaling problem has been considered elsewhere⁴, and it has been shown that the much larger viscosity of liquid magmas is compensated for by the larger scale, so that the laboratory and prototype flows can be dynamically similar. We have drawn attention to a previously neglected fluid process, that of upflow in the boundary layer accompanying crystallization of a heavier component at a side boundary. This is in principle capable of setting up large scale concentration and density gradients through the depth of a magma chamber, and in combination with double-diffusive processes, it can also produce smaller scale layering. It seems at least worth comparing the consequences of this model with some of the detailed petrological and geochemical data on igneous intrusions.

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Evidence from Canada and Alaska on plate tectonic evolution of the Arctic Ocean Basin

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A new model for the plate tectonic evolution of the Amerasian sector of the Arctic Ocean is suggested here using data from petroleum exploration on its margin. More than 500 km of late Cretaceous dextral slip is indicated for the SW–NE trending Kaltag fault system in Alaska and northwestern Canada. Terminating against the Kaltag, an older fault system trends along the Canadian Cordillera, with dextral slip of thousands of kilometres. Its offset by the Kaltag is postulated to follow the southern margin of the Canada Basin. Restoration of the offset creates a great circle lineament from the Asian end of the Lomonosov Ridge through western North America along which Jurassic–Cretaceous transform movement could have accommodated spreading about the Alpha Ridge. Accretion of the newly-formed lithosphere to a Palaeozoic Canada Basin created the Amerasian Basin in essentially its present configuration, modified by subsequent Kaltag movement.

The Arctic Ocean is divided into the Eurasian and Amerasian basins, separated by the Lomonosov Ridge. Proponents of seafloor spreading have generally agreed that the Eurasian Basin originated through Cenozoic spreading about the Nansen (Gakkel) Ridge¹. The origin of the Amerasian Basin, bounded by the Lomonosov Ridge and the Arctic continental margins of North America and Siberia, is more controversial^{2,3}. Petroleum exploration on the continental shelf and mainland of Alaska and northern Canada provides new data on this problem. The Richardson Mountains of Northwestern Canada are part of a broad shear zone flanked on the west by the Kaltag fault system (Fig. 1), whose principal movement was during the late Cretaceous⁴. Local and regional facies changes in Lower and Upper Cretaceous sediments occur between adjacent fault blocks^{5,6}, suggesting an aggregate dextral slip for the entire fault and shear zone of several hundred kilometres. The youngest strata involved in faulting are of Maastrichtian and possible Palaeocene age⁷. Offshore geophysical surveys and deep drilling have delineated an Upper Cretaceous–Tertiary sedimentary basin containing >8 km of regressive clastic sediments^{8,9}. The oldest sediments drilled so far are of Palaeocene age. The sediments are deformed, but the structures are synsedimentary and do not seem to be affected by the underlying belt of strike-slip faults, implying that those movements had ceased in

the early Tertiary or latest Cretaceous.

The Kaltag fault system extends from western Alaska through northwestern Canada and passes northeastward beneath the Beaufort Sea^{9,10}. It separates east–west trending structures of the Brooks Range from the Interior Platform and from the eastern part of the Canadian Cordillera, represented locally by the Mackenzie Mountains. From the Beaufort Sea, it is believed to follow the linear continental margin of the Canadian Arctic Archipelago^{4,6,11}, with minor offset by the Wegener fault, to Greenland. The trend is continued along the southern margins of the Eurasian Basin and Barents Shelf, forming a lineament 4,500 km long.

South-west of the Richardson Mountains, the Kaltag fault intersects the Tintina fault (Fig. 2). Following the trend of the Canadian Cordillera, the Tintina fault is a major strike-slip fault whose post-Palaeozoic dextral slip, based on offsets of equivalent strata, is at least 450 km¹². It is one of several dextral strike-slip faults, sub-parallel to one another, closely associated with ophiolite zones and areas of blueschist metamorphics, trending through the Western Cordillera of North America to the America landfall of the East Pacific Rise^{13,14}. Palaeolatitudes of Palaeozoic rocks west of this zone are several thousand kilometres south of palaeolatitudes of coeval strata in the North America craton and eastern part of the Cordillera¹⁵. Actual displacements of the Tintina and associated faults are probably much greater than the measured offsets.

The Tintina fault terminates at its northern end against the Kaltag fault. It is postulated that it was displaced 560 km northward by the Kaltag, and that the offset continuation is represented by the southern margin of the Canada Basin, an abyssal plain underlain by oceanic lithosphere^{8,16}. Several workers have speculated on the presence of a fault bounding the Alaskan margin of the basin^{11,17}. It is suggested that such a fault, herein designated the Beaufort–Laptev fault, follows the southern margin of the Amerasian Basin from the Beaufort to the Laptev Sea, at the Asian end of the Lomonosov Ridge. Although the margin is not as linear as some fault-controlled ocean margins, the irregularity may be due to modification by volcanism and sedimentation, and by the anticlockwise rotation of the sector east of long. 180° implicit in the Kaltag fault movement.

Restored to their Mesozoic continuity (Fig. 2), the Beaufort–Laptev and Tintina faults formed part of a lineament that followed a great circle from the Asian end of the Lomonosov Ridge via the Western Cordillera of North America to the landfall of the East Pacific Rise. It is suggested that this lineament was a Mesozoic transform fault zone through which Pacific seafloor spreading was transferred to spreading about the Alpha Ridge, a Mesozoic analogue of the Cenozoic fault system connecting spreading about the Nansen Ridge with opening of the North Atlantic. Major movements along this lineament ceased when the Tintina and Beaufort–Laptev sectors became separated at the end of the early Cretaceous. Subsequent Pacific seafloor spreading was perforce accommodated by subduction beneath the Aleutian Arc.

Relative to localities in North America, the apparent polar wander (APW) path shifted abruptly at the end of the Lower Cretaceous (100 Myr)¹⁵, the time when the offset of the Tintina–Beaufort–Laptev lineament by the Kaltag fault was initiated. Although APW paths do not indicate absolute movements of the continents relative to a magnetic north pole, it is surely more than coincidence that the angular shift of the APW path is similar to the angle between the Tintina and Kaltag faults.

Spreading about the Alpha Ridge during the Jurassic and early Cretaceous created a large trapezoidal ocean basin which, through accretion to the pre-existing Canada Basin, gave rise to the Amerasian Basin in almost its present form, modified by dextral movement of its eastern margin in the late Cretaceous.

Major discontinuities of Phanerozoic sedimentary and metamorphic facies coincide with both the Kaltag and the Tintina faults^{6,18}. Maps of Mississippian through Upper Cretaceous facies can be redrawn palinspastically to relate

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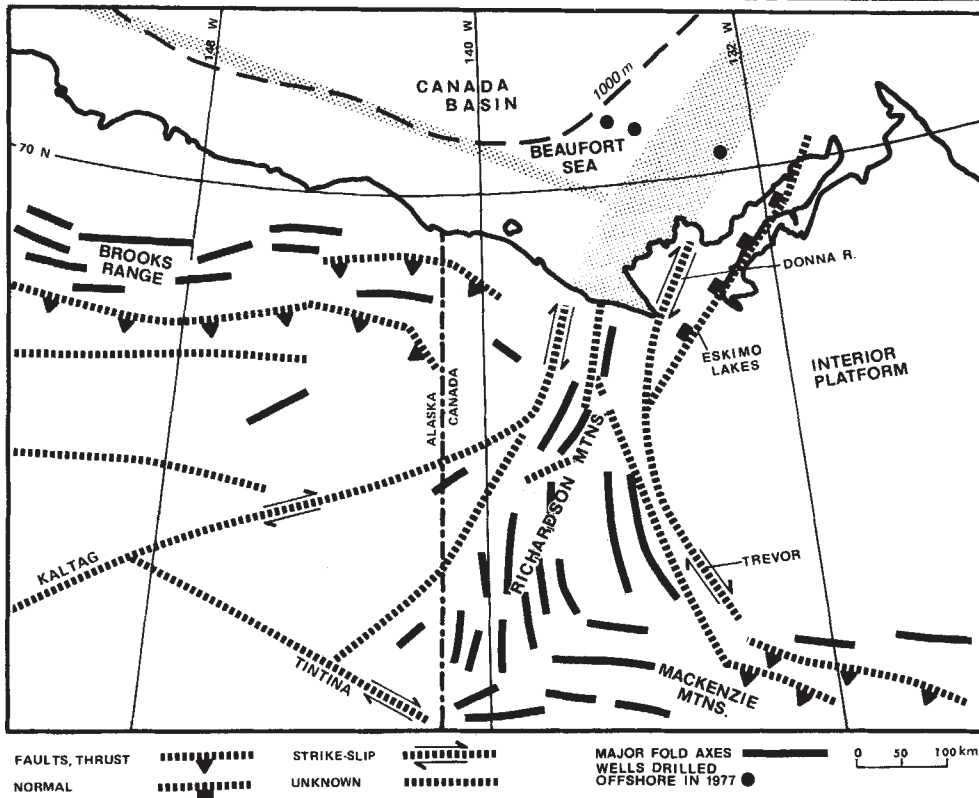
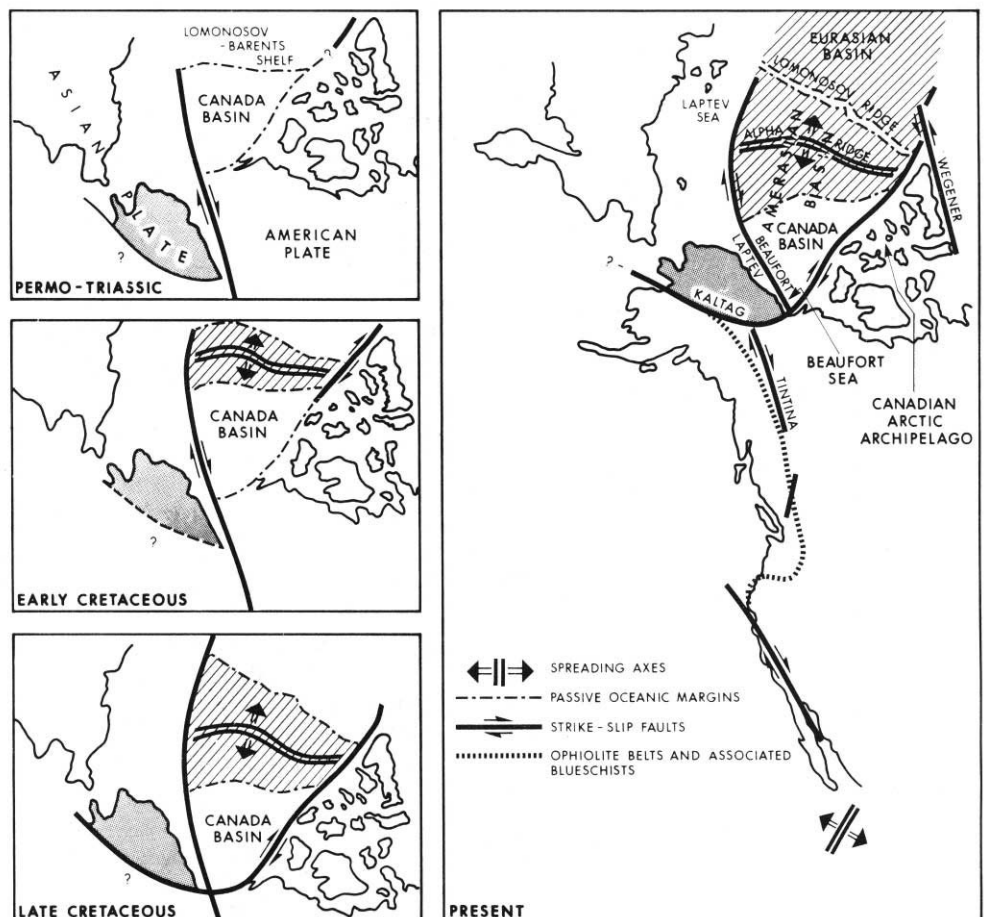


Fig. 2 Model for the evolution of the Amerasian Basin and associated displacements along Tintina and KALTAG faults.



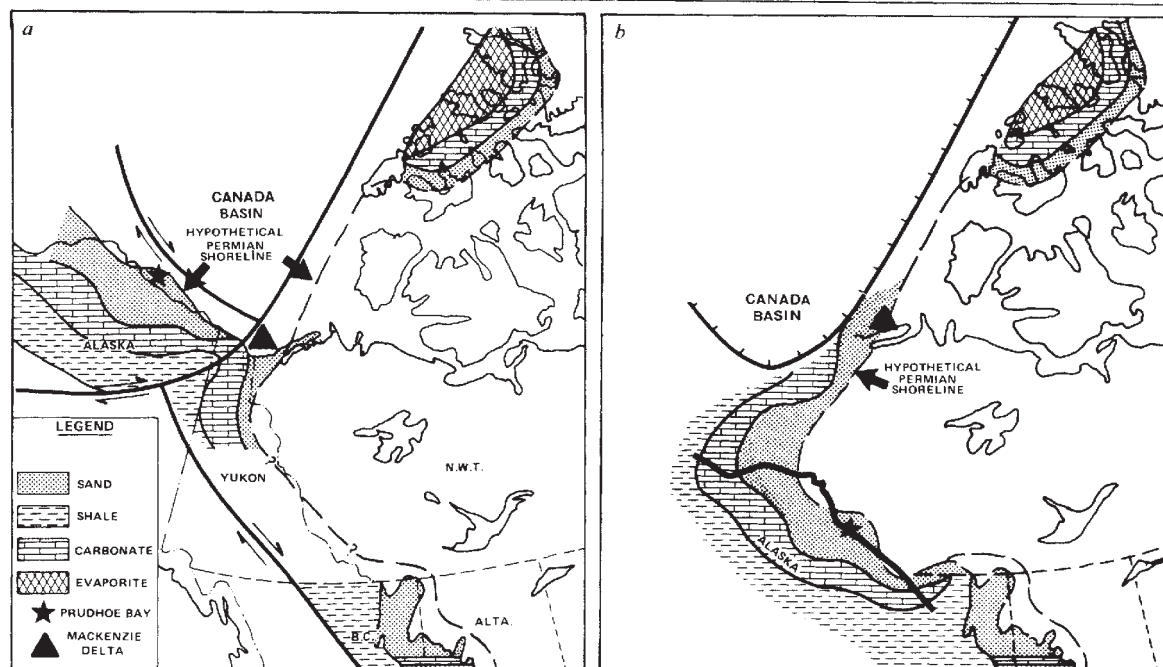


Fig. 3 a, Present distribution of Permian lithofacies (Modified after ref. 18). b, Palinspastic restoration of facies according to fault movements shown in Fig. 2.

movements of these faults to the evolution of Upper Palaeozoic and Mesozoic palaeogeography of northwestern America. The restoration of Permian facies (Fig. 3) is used as an example, since it provides a new solution to the problem of provenance of Permo-Triassic sandstones of northern Alaska, reservoir rocks in the giant Prudhoe Bay oilfield. These sediments were derived from an adjacent land mass in the area now occupied by the Canada Basin^{19,20}, leading many workers to conclude that their source was the Canadian Arctic Archipelago, split off from Alaska by Jurassic opening of the Canada Basin²¹⁻²³. Figure 3 shows a common sediment source for the Permian of both northern Alaska and the Canadian Cordillera. Matching of facies across the Tintina fault entailed restoration of 1,200 km of Jurassic-Early Cretaceous dextral movement, a distance that corresponds approximately with the width of oceanic lithosphere produced by seafloor spreading about the Alpha Ridge.

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Large historical earthquakes and seismic risk in Northwest Syria

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Arabic documents and manuscripts containing detailed descriptions of material damage and ground deformation due to earthquakes in northwestern Syria have been used to establish a catalogue of local seismicity and assign epicentral intensities to the earthquakes. Large earthquakes affecting the northern Syrian fault system apparently have a recurrence interval of about 340 ± 60 yr. Here we use this figure and a relationship between recurrence interval and seismic moment, recently proposed by Molnar, to estimate the maximum seismic moment ($0.5 \times 10^{27} \leq M_0^{\text{max}} \leq 3.7 \times 10^{27}$) and the magnitude ($7.1 \leq M_w \leq 7.7$) of the largest earthquakes in northwestern Syria. Such an earthquake is not expected for at least one century but an earthquake with magnitude $6 \leq M_w \leq 7$ might occur in the near future.

The great linear fault system which begins near the Dead Sea rift and continues, north of Lebanon, by the Syrian faults has exhibited only a moderate seismicity since earthquakes have been instrumentally recorded (since the turn of the century).

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However, archaeological and historical documents show that major earthquakes have affected Palestine and Syria in earlier times. Many biblical events can be interpreted as great earthquakes¹ affecting the Dead Sea system. Further north, Antioch, once the capital of Syria (now Antakya in Turkey) and one of the great cities of the Mediterranean ancient world was repeatedly destroyed by terrible earthquakes². Ambrasey³ showed, and it is now generally accepted, that the instrumental record is too short to give an unbiased image of the seismicity of a given area and that historical records must be used. Thus, for large shallow earthquakes at plate boundaries, it has been possible to define recurrence intervals for large earthquakes around the Pacific and in the Caribbean⁴ and forecast that shocks should occur in the near future in areas for which the recurrence interval was nearly the length of time elapsed since the last large shock. Nur and Reches¹ have recently found a recurrence interval of 200 yr for 6–7 magnitude earthquakes on the Dead Sea system.

From Arabic manuscripts and documents, Taher⁵ has gathered data on historical earthquakes in the Near Middle East from the seventh up to the eighteenth century. Clearly, many earthquakes essentially affected the region of Antioch, Aleppo, the Border zone (north of Antioch along the Turkish border) and the Orontes Valley (Hama, Homs, and so on). This region corresponds to the area north of Lebanon, threaded by the Syrian fault system which comprises the straight Masyaf fault, the Gharb and Antioch grabens and the Karasu fault (Fig. 1). There is field evidence⁶ that the whole system functions as left lateral strike-slip faults, thus constituting the northern part of the transform fault bounding the Arabian plate on the west, the southern part of which forms the Dead Sea system which has been shown to be the seat of a sinistral strike-slip motion^{1,7}. The northern and southern parts of the fault system are separated by the compressive Lebanon region and can be considered as different rupture zones. It is, therefore, reasonable to concentrate on the seismic activity of the Syrian fault system alone. Accordingly we catalogued local seismicity (Table 1) using data from: Greek and Roman historians² from AD 52 to 588; Taher⁵ for the period covered by arabic texts (seventh–eighteenth century); Karnik⁸ for the nineteenth century and instrumental seismicity up to 1951; the ISC Bulletin (Edinburgh) from 1951 to the present.

We considered only the meizoseismal zone in the region of Antioch, Aleppo and the Orontes Valley. Epicentral intensities I_0 , on the Mercalli modified scale, have been assigned to the earthquakes by using the description of damage given in the documents.

We find essentially two classes of earthquakes: the ones with $VII \leq I_0 \leq X$ and the ones with $I_0 \geq X$ for which there is evidence of ground deformation of tectonic origin and/or particularly great damage. We did not include the shocks with $I_0 < VII$ nor those with $M_L < 5$. The spectrum of epicentral intensity I_0 versus time displays a rather regular pattern with two recurrence intervals (Fig. 2): Large earthquakes ($I_0 \geq X$) occurred in AD 115, 528, 859, 1139 and 1822 with a recurrence interval:

$$T_1 = 341 \pm 62 \text{ yr}$$

smaller earthquakes ($VII \leq I_0 < X$) occur with a recurrence interval:

$$T_2 = 71 \pm 51 \text{ yr}$$

Quakes with $I_0 \geq VII$ reported at dates differing by < 5 yr were counted as one in the average). Although the earthquake of AD 115, under Trajan was very severe, the evidence to assign it an intensity $I_0 \geq X$ is not as good as for the others; however, if the unmistakable quasi-periodicity is to have a physical meaning, the earthquake of AD 115 should be included. A gap in the spectrum for smaller earthquakes between 1408 and 1822 is attributed not to the lack of seismicity but to the lack of chroniclers in Damascus under the Ottoman rule: only the great earthquakes felt in a wide area would have been recorded.

Molnar⁹ recently estimated the average recurrence interval T

of events with seismic moments $\geq M_0$ but not greater than $M_0^{\max}$ (the seismic moment of the largest earthquakes which have occurred on the fault):

$$T = \frac{(M_0^{\max})^{1-\beta} M_0^\beta}{(1-\beta) \dot{M}_0^\beta}$$

where $\beta \approx \frac{2}{3}$ and where $\dot{M}_0^\beta$ is the average production rate of seismic moment given by:

$$\dot{M}_0^\beta = \mu l L \bar{V}$$

where μ is the rigidity of the crust; l , the width of the fault; L , the active length of the fault system and $\bar{V}$, the average rate of motion along the fault.

We will use Molnar's formula, not to estimate the recurrence intervals from the knowledge of the seismic moments but the other way round, for the following reasons: our historical evidence refers to local earthquakes, and all reports of damage refer to the same geographical area, hence, we can compare the quakes on the basis of the importance of the damage and of the ground deformation. We therefore assume that the earthquakes to which we have assigned the maximum epicentral intensity on this basis (and which have a recurrence interval $T_1 \approx 341$ yr) are the ones which have the maximum seismic moment $M_0^{\max}$.

We can now estimate $M_0^{\max}$ from Molnar's formula, assuming reasonable values for the parameters β , μ , l , L and $\bar{V}$.

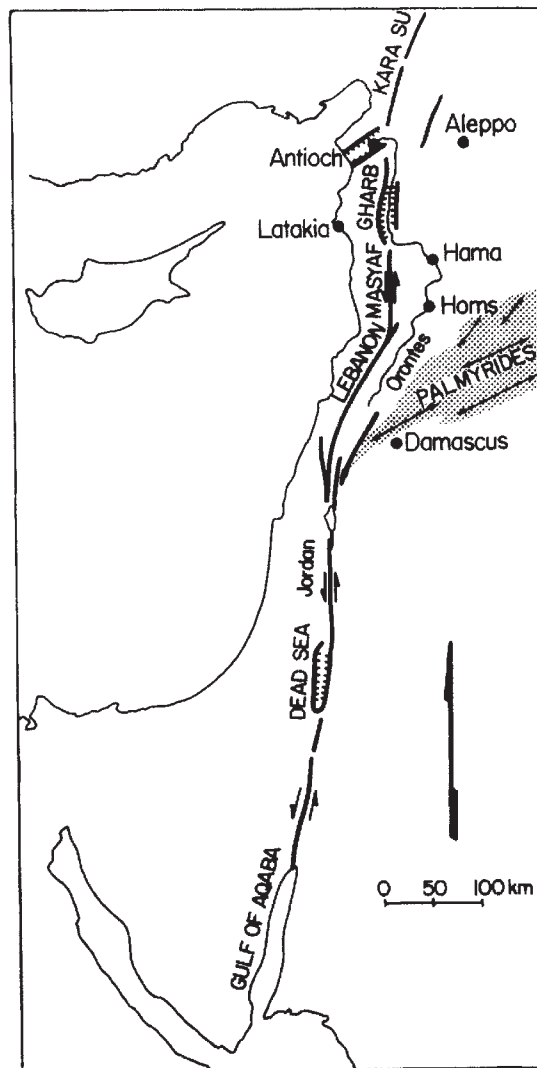


Fig. 1 Simplified map of the Dead Sea–Syrian faults system.

Table 1 Historical and instrumental seismicity

Date (AD)			I_0	M_L
52	Antioch	Destructions	VIII-IX	
115	Antioch	Heavy destructions	X-XI	
340	Antioch	Heavy destructions	IX	
394	Antioch	Heavy destructions	IX	
396	Antioch	Heavy destructions	IX	
458 September	Antioch	80,000 victims	IX	
526 May 29	Antioch	Very severe earthquake	IX-X	
528 November 29	Antioch	A mountain fell into the Euphrates at Quludhya, the Euphrates shifted its bed	X-XI	
581	Antioch	The suburb Daphne was destroyed	VIII-IX	
588 October 31	Antioch	Destroyed, 60,000 victims	IX	
634	Aleppo	Ramparts and fortress destroyed	VIII	
713 March 20	Antioch	Completely destroyed	IX	
859 December	Antioch	1,500 houses destroyed, 90 towers fell from the ramparts, Jebel al Aqra' (Bald mountain or Mons Casius 30 km SW of Antioch, close to the sea, 1,759 m high) "fell into the sea". A river disappeared into the ground; Urfa, Adana, Tarsus, Misis, Homs, Damascus destroyed	X-XI	
951 September	Aleppo	Heavy destructions, Ra'ban, Duluk (fortress between Aleppo and Antioch) destroyed	VIII-IX	
972	Antioch	Emperor Johannes Shamschik send 12,000 workers to rebuild the city	IX	
1002	Syria	Border zone, many destructions	VIII-IX	
1063 July	Antioch	Latakia, Tripolis, Acre	VIII	
1091 September 17	Antioch	70 towers fell from the ramparts	IX	
1139 November	Aleppo	Destroyed, evacuated by the inhabitants "The earth shook and the stones shook like grain in the winnowing basket"	X-XI	
1156-1159	Orontes	Aleppo, Hama, Afameya (Apamea), Shayzar (Larissa) Homs (Emessa)	IX-X	
1170 June 30	Aleppo	Totally destroyed, 80,000 victims; Damage in Orontes Valley	IX-X	
	Antioch	St Peters cathedral collapses over the patriarch		
1343 January 1	Membij	Destroyed, 5,700 victims, Aleppo fortress destroyed	IX	
1404 February 11	Aleppo	Heavy destructions, Latakia fortress destroyed	IX	
December 5	Aleppo	Three shocks	VII	
1408 December 30	Antioch	Heavy destructions, the ice fell off the top of Jebel al Aqra'	X-XI	
	Aleppo	Heavy destructions. Fissure 1 mile long in the neighbourhood (between al Qucir and Saltuhum)		
1822 August 13	Latakia	Sea wave		
	Antioch	Aleppo destroyed at 60%	X-XI	
September 5	Aleppo	Sea wave at Iskenderun		
	Aleppo	Destruction of what remained, 20,000 victims		
1872 April 2	Antioch	Destroyed at 30%, 500-1,800 victims		
1936 June 14	Epicentre	near Antioch (36.5° N, 36° E)	VII	5.5
1951 April 8	Epicentre	near Antioch (36.6° N, 36° 3'E)	VII	5.7
1971 June 29	Epicentre	100 km of Aleppo (37.2° N, 36.8° E)		5
July 11	Epicentre	100 km of Aleppo (37.1° N, 36.9° E)		5

We take $\beta = 0.61$ from the empirical relationship found by Chinnery and North¹⁰ for the whole world:

$$\log N = 17.47 - 0.61 \log M_0$$

where $N = 1/T$ is the frequency of the earthquakes having a seismic moment $\geq M_0$. This relationship is the same as the one on which Molnar's formula rests: $N = \alpha M_0^{-\beta}$, with $\beta = 0.61$.

We take the usual value for the rigidity of the crust: $\mu = 3.3 \times 10^{11}$ dyn cm⁻².

The depth l of the strike-slip faults is the depth of the brittle crust. Crustal structure studies by Ben Menahem *et al.*¹¹ in the Jordan area give $l = 20$ km. We will adopt this value.

We will take for L , the length of active fault, the total length of the fault system north of Lebanon, including the Karasu fault: $L \approx 300$ km. Although the fault system becomes more diffuse in the Antioch area this does not prevent us applying the formula⁹.

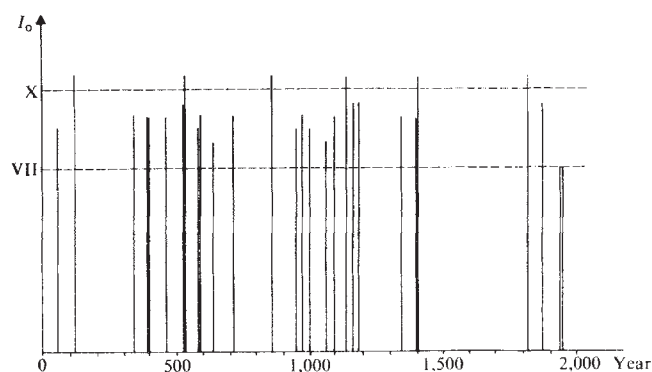


Fig. 2 Spectrum of the intensity of earthquakes in North-west Syria from the first century AD to present. The Mercalli modified intensity in the epicentral zone has been assigned from the description of damage and ground deformation (summarized in Table 1).

The value of $\bar{V}$ relative displacement velocity along the fault (rate of slip) can be calculated from the present day relative motion of the Arabic and African plate given by Minster and Jordan¹²; their results were obtained by inverting data for the whole world (among which the Red Sea and Gulf of Aden rates of expansion): the value found, $\bar{V} = 1.2$ cm yr⁻¹ is probably a little high, as part of the displacement along the transform fault is absorbed by deformation of the Lebanon and the Palmyrides and by some aseismic slip¹¹. We will take $\bar{V} \approx 1$ cm yr⁻¹ (On geological evidence, Freund *et al.*⁶ have found a value for v of the order of 0.5 cm yr⁻¹ in the Dead Sea region).

The uncertainties in these parameters and in the recurrence interval make up those inherent to Molnar's formula (which we will not take into account) to make our estimate of $M_0^{\max}$ rather vague.

Assuming that the minimum value of $M_0^{\max}$ corresponds to: $T_1 = 279$ yr, $l = 10$ km, $\bar{V} = 0.5$ cm yr⁻¹ and the maximum value to the choice: $T_1 = 403$ yr, $l = 20$ km, $V = 1.2$ cm yr⁻¹, we find:

$$5.4 \times 10^{26} \leq M_0^{\max} \leq 3.7 \times 10^{27} \text{ dyn cm}$$

We can apply Kanamori's formula¹³:

$$M_w = \frac{\log M_0}{1.5} - 10.7$$

We obtain for the largest earthquakes recurring at the rate of one every 341 ± 62 yr:

$$7.1 \leq M_w \leq 7.7$$

The preferred value corresponds to $T_1 = 341$ yr, $l = 20$ km, $\bar{V} = 1$ cm yr⁻¹; it is:

$$M_0 \approx 2.6 \times 10^{27} \text{ dyn cm}$$

$$M_w \approx 7.6$$

Having an estimate for $M_0^{\max}$, we can now estimate the seismic moment of the smaller earthquakes with a recurrence interval $T_2 = 71 \pm 51$ yr. We find:

$$2.5 \times 10^{25} \leq M_0 \leq 4.9 \times 10^{26}$$

or

$$6.2 \leq M_w \leq 7.1$$

with a preferred value:

$$M_0 \approx 2 \times 10^{26}$$

$$M_w \approx 6.8$$

This agrees with the figure given by Ben Menahem *et al.*¹¹ for the Jordan Valley where they find an average of two events per century at magnitudes from 6 to 7.

Our catalogue of local seismicity and the application of Molnar's formula suggests that there are two different recurrence interval for earthquakes in North-west Syria due to the operation of the Syrian fault system: Earthquakes with $M_w \approx 7.6$, the largest for the region have an average recurrence interval of 341 ± 62 yr; smaller earthquakes with M_w between 6 and 7 occur

with a recurrence interval of 71 ± 51 yr. The last large earthquake occurred in 1822, the next one is, therefore, not due for at least one century. On the other hand, as there has been no destructive earthquake since 1872, we forecast that an earthquake with a magnitude between 6 and 7 should occur soon.

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Sinistral movement along the Gulf of Aqaba — its age and relation to the opening of the Red Sea

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The Red Sea forks northwards into the Suez Graben and the Dead Sea Arava Rift (Fig. 1). The southern part of the latter, about 165 km long, is occupied by the Gulf of Aqaba (Elat). A sinistral strike-slip movement of about 107 km which was suggested to have occurred along the Dead Sea–Arava Rift^{1–3} is related to the opening of the Red Sea proper^{4–6}. The opening of the Red Sea was considered to have taken place in two stages. The various dates proposed for the first movement are: from 41 to 34 Myr (ref. 5), from 29 to 24 Myr (ref. 7) and from 20 Myr onwards⁶. The second movement is generally dated from 4–5 Myr to Recent. Garfunkel and Bartov⁸ claimed that the rifting in the Gulf of Suez was well under way in the span of the late Oligocene to early Miocene (30–20 Myr) and that a drastic change in the tectonic regime occurred in the middle Miocene (15–16 Myr). We suggest here, however, that the opening of the Red Sea is younger than 19–22 Myr.

Initiation of faulting within the Gulf of Aqaba⁹ is assumed to predate the Neogene Raham Conglomerate which was deposited in a morphotectonic depression with structural relief exceeding 1.2 km. It was suggested that the first stage of horizontal movement began in the Mesozoic and resulted in a total displacement of about 60 km, and that a further movement of about 40–45 km was of post-Miocene age¹⁰. Freund and Garfunkel¹¹ suggested that the first stage of slip along the Dead Sea occurred in the early Miocene or earlier, and the second stage in the late Miocene or in the Pliocene. A different approach¹² suggested that the whole 107-km shear along the Dead Sea–Arava Rift may have occurred in the middle Miocene or later.

A belt of anastomosing faults was mapped by various investigators^{3,13,14} in eastern Sinai, along the Gulf of Aqaba. This belt is part of the Dead Sea–Arava Rift Valley system. Small sinistral

displacements, from hundreds of metres to several kilometres, were reported in this belt^{3,11,13,15–17} based on offsets of lithological contacts in the Precambrian terrain or on geometric considerations pertaining to deformed strata of the sedimentary cover (Cambrian to Eocene). Freund and Garfunkel¹¹ suggested that these faults were active primarily, or only during the first stage of slip. Eyal¹³ and Ben-Avraham *et al.*¹⁸ noted that these faults did not participate in the youngest movements. Young faulting in this sector of the Rift is recorded only from within the Gulf and its shores¹⁸. In the present study we attempt to define more precisely the shear and its age along the western margin of the Gulf of Aqaba. This is a continuation of dating the movement on the Central Sinai–Negev Shear Zone^{12,19}.

Long basalt and olivine basalt dykes, trending SE–NW have recently been identified in southern Sinai (Fig. 1). These dykes are up to 100 m wide, tens to hundreds of kilometres long and are spaced several tens of kilometres apart. Some intrude Mesozoic sediments. They are similar to Tertiary igneous rocks which have been described in the Eastern Desert of Egypt^{20–22}, the Gulf of Suez⁸, Central Sinai¹⁹ and Saudi Arabia²³, all of which are considered to be of Miocene age based on field relations and K–Ar dating^{12,24–26}. The dykes in southern Sinai have been dated by the K–Ar method. Results on five bodies yield ages of intrusion of 18–22 Myr which is a distinct and widespread volcanic phase in the Red Sea area.

Figure 1 presents the fault pattern along the Gulf of Aqaba. Most of the faults are in the form of lineaments within the Precambrian terrain. The general trace of the fault belt along the Gulf is of arcuate form.

The amount of sinistral movement along these faults was determined by offsets of magmatic bodies and lithological contacts in rocks of Precambrian age. These offsets were compared with offsets of the early Miocene dykes, on the same faults. In the areas where detailed mapping was undertaken identical displacements were recorded by both groups of markers.

In area A (Fig. 1) near Bir Zreir, the total cumulative displacement across the whole fault belt attains some 24 km. In most cases the amount of displacement on single faults is 0.8–4.0 km. The largest sinistral displacement recorded on a single fault in this area is 9.8 km. As the amounts of displacement measured on the Precambrian markers are in good agreement with those indicated by the early Miocene dykes, we suggest that the total 24 km of shear along this fault system postdates the early Miocene volcanic phase.

Faults showing a similar geometric pattern are recognized on the LANDSAT images of the Saudi coast of the Gulf of Aqaba

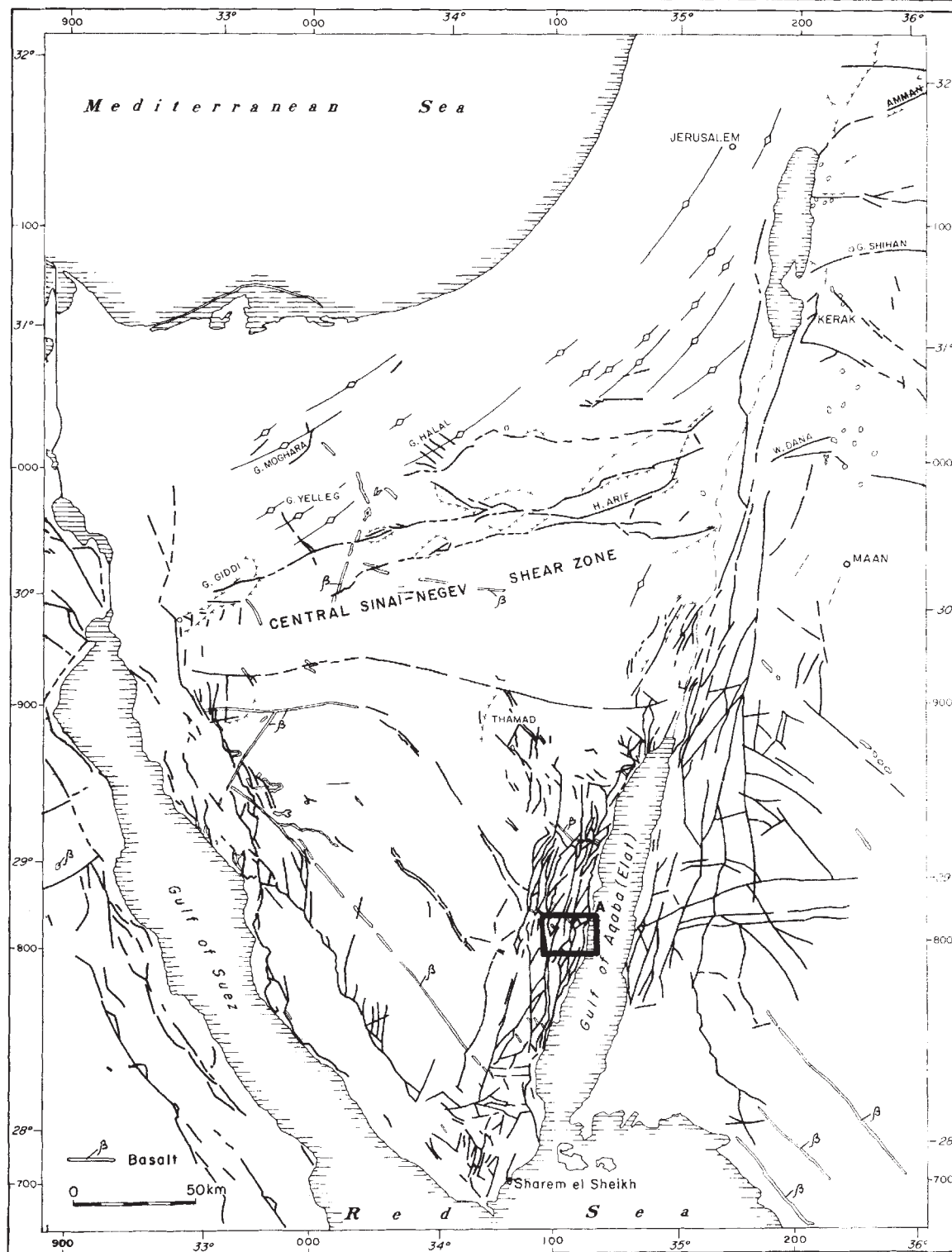


Fig. 1 Early Miocene volcanism and the Tertiary to Recent fault pattern in Sinai and adjoining areas. A, Bir Zreir area.

(Fig. 1). Some of these were mapped by Bramkamp *et al.*²⁷, Bender²⁸ and Freund *et al.*³. The amount of sinistral movement which is observable along these faults is at least some 18 km (based on displaced Precambrian elements that could be traced on the LANDSAT images). It is assumed that the total sinistral movement along the eastern side of the Gulf is approximately the same as that observed along the western margins of the Gulf of Aqaba. Thus only about 60 km of the total 100–105 km movement has been taken up by faults within the Gulf itself.

The analysis of the fault belt area along the Gulf of Aqaba suggests that the intrusion of very long basaltic dykes in a NW–SE direction, parallel to the coasts of the Red Sea and the Gulf of Suez, accompanied the initial stage of the development of the Red Sea. The igneous activity can probably be related to the faulting which led to the clear demarcation and subsidence of the Suez Graben⁸ and the Red Sea²⁹. Furthermore, some of the magnetic anomalies recorded in the Red Sea and in areas marginal to the Red Sea³⁰ may be related to such dyke intrusions.

A drastic change in the geotectonic regime occurs after the intrusion of the early Miocene dykes. We suggest that the large-scale horizontal movements (107 km) recorded along the Dead Sea–Arava Rift, postdate the intrusion of the early Miocene dykes, that is, are younger than 19–22 Myr. The movement along the Dead Sea–Arava reflects the seafloor spreading in the Red Sea. The age relations proved for the Dead Sea–Arava Rift imply that the opening of the Red Sea is also younger than 19–22 Myr.

Several authors have tried to relate the tectonic pattern of Israel and adjacent countries to the horizontal shear along the

Gulf of Aqaba–Dead Sea Rift^{4,31,32}. Considerable pre-Miocene tectonic activity is known along the Syrian Arc system³³. Because of the age differences this activity cannot be considered as resulting from the shear along the Dead Sea–Arava Rift. This does not exclude the possibility that elements of the Syrian Arc and the Dead Sea Rift may have shown some slight initial activity as early as the Senonian^{34,19}.

Future work aimed at defining and correlating individual Neogene basaltic bodies on both sides of the Rift could help to establish an exact fit across the Rift.

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Coccoliths in Pleistocene–Holocene nannofossil assemblages

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Marine palaeotemperature studies are increasingly using oxygen isotope analyses of the minute calcium carbonate structures produced by a group of marine phytoplankton, the coccolithophores^{1–6}. To provide a sound experimental basis for palaeotemperature calculations using the isotopic data from analyses of coccoliths, we have grown coccolithophores in laboratory batch culture in controlled environmental conditions, and determined the oxygen isotopic compositions of the coccoliths produced at known temperatures. The results reported here indicate that the oxygen isotopic composition of the coccoliths of all the species studied is strongly temperature dependent. A 'vital effect' was observed in all the species, with the isotopic values of different culture samples falling into two definite groups containing separate taxa. The difference in vital effect between different species suggests that calcification processes may vary among different taxa and indicates that a re-evaluation of coccolith oxygen isotope palaeoclimatic interpretations may be in order.

Coccolithophores possess certain unique advantages for oxygen isotope palaeoclimatic studies. As phytoplankters, they are restricted to the photic zone, with maximum population densities found between 50 and 100 m depth in most oceanic areas⁷. Consequently, coccolith secretion takes place only at near-surface water temperatures. On the other hand, planktonic foraminifera may produce their tests at the various temperatures found between the surface and depths as great as 1,500–2,000 m, although depth ranges for individual taxa may be more narrowly restricted⁸. In addition, coccolithophores show a wide geographic distribution, being found in all but polar seas⁷. Finally, coccoliths are more abundant than foraminiferal tests in many Tertiary and Cretaceous marine sediments⁹.

We have maintained in laboratory culture the previously isolated *Emiliania huxleyi*, and successfully isolated and cultured for the first time *Gephyrocapsa oceanica*, *Cyclcoccolithus leptoporus*, and *Thoracosphaera heimii*. These four species produce coccoliths frequently encountered in many Pleistocene and Holocene sediments.

The clonal algal cultures were grown in continuous batch culture and acclimated for 2 to 3 weeks at each temperature (12, 16, 20, 24, and 28 °C) before the coccoliths of the last batch culture were collected for analysis. The cultures were grown in 33‰ seawater enriched with f/2 levels of nutrients as described by Guillard¹⁰ with silicic acid omitted. Light was provided by cool white fluorescent bulbs producing 0.023 langley min⁻¹. Coccoliths were concentrated by centrifugation in buffered distilled water, then oven dried and roasted under vacuum at 400 ± 25 °C to remove residual organic matter. The carbonate samples were reacted at 50 °C according to the method developed by Shackleton^{11,12} and analysed by mass spectrometry. Samples of the water of the growth medium were analysed according to the method of Epstein and Mayeda¹³ and corrections made according to Craig^{14,15}.

Analytical data obtained from over 70 samples showed two important trends. The $\delta^{18}\text{O}$ values of the coccoliths of *G. oceanica* and *E. huxleyi* are strongly temperature dependent

(Fig. 1), but are ~1‰ positive (enriched in ^{18}O) relative to calcium carbonate precipitated at equilibrium. The $\delta^{18}\text{O}$ values obtained from coccoliths of *C. leptoporus* and from tests of *T. heimii* also show a strong temperature dependence but are ~2.5‰ depleted in ^{18}O relative to equilibrium (Fig. 1). The isotopic compositions of these latter species are similar to values reported for coccoliths of *Cricosphaera carterae*⁵. Cultures of *G. oceanica* were maintained under both 14:10 hour light/dark cycles and 24-h light regimes. The $\delta^{18}\text{O}$ data on this species (Fig. 1) indicate no significant difference in oxygen isotopic composition related to the period of illumination.

The experimental data show a temperature dependence in oxygen isotopic composition which in no case corresponds to equilibrium precipitation of calcium carbonate. This would indicate that in all the coccolithophore species studied, a not unexpected vital effect influences the fractionation of oxygen

photosynthetic O_2 . Much work obviously remains to be done to understand the process of calcification in these planktonic algae. Relatively little is known about pathways, although our data do imply that calcification processes in coccolithophores may vary among different taxa. This is further suggested by the close grouping of the isotopic data for *E. huxleyi* and *G. oceanica* (Fig. 1), as these two species are thought to lie in a phylomorphogenetic lineage²⁴.

Recently, several investigators^{3,4} have published analyses of 'bulk' carbonate samples composed of mixtures of different coccolith species and small foraminiferal tests. The isotopic signal recorded in such samples is a complex mixture of the isotopic signals of the different species comprising the bulk sample. Apparently the isotopic signal cannot be interpreted without data on the vital effect of each species included.

The presence of a vital effect in the fractionation of oxygen isotopes in coccolithophores does not preclude the use of coccoliths for oxygen isotope palaeotemperature analysis, as long as the magnitude of the effect is known over the temperature range in question. Nanofossil assemblages composed of coccoliths of *E. huxleyi* and *G. oceanica*, both of which are small, may be separated by settling and filtering techniques, and analysed for the oxygen isotopic composition. The $\delta^{18}\text{O}$ values of such an assemblage reflect the temperature and the $^{18}\text{O}/^{16}\text{O}$ ratio of the water in which these coccolithophores lived, even though the mineral was not precipitated in equilibrium. Factors such as light intensity, nutrients, and salinity may also play a role in regulating fractionation. These variables and their effect on isotopic composition are being examined in our laboratories.

Our increased understanding of the vital effect in the fractionation of oxygen isotopes by coccolithophores should increase the value of coccolith oxygen isotopic data in palaeoclimatic studies. The $\delta^{18}\text{O}$ data from coccoliths used in conjunction with oxygen isotopes data from foraminifera should allow us to determine with greater reliability variations in surface water temperature during the Pleistocene.

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Note added in proof: The taxonomic position of the *Thoracosphaera*, that is, whether they are coccolithophores or dinoflagellates, has been a matter for debate. Based on detailed examination of cell ultrastructure and particularly the presence of a typical dinoflagellate nucleus, we have concluded that *Thoracosphaera heimii* is a dinoflagellate and not a coccolithophore (L.E.B., P.L.B. and R.L.G., manuscript in preparation).

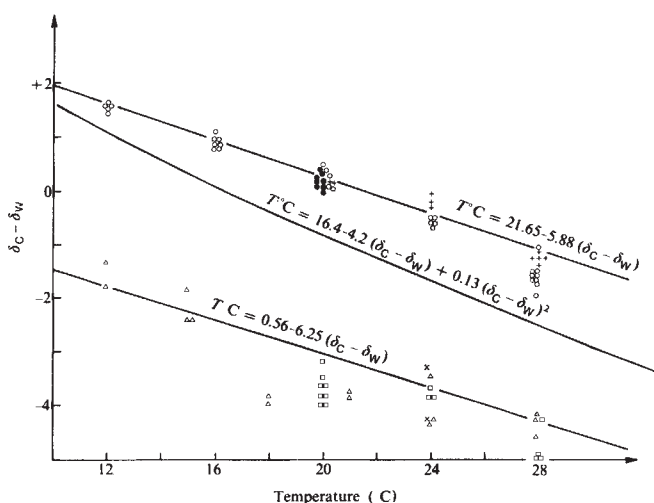


Fig. 1 The species plotted are *E. huxleyi*, 14:10 h light/dark cycle (+); *G. oceanica*, 14:10 h light/dark cycle (○), 24-h illumination (●); *C. leptoporus* 14:10 h light/dark cycle (□). *T. heimii*, 14:10 h light/dark cycle (X); and *C. carterae*⁵, 12:12 h light/dark cycle (△). Each datum point represents an individual unialgal batch culture. The isotopic composition of the coccoliths are reported as $\delta_C - \delta_W$ versus temperature, where $\delta_C = \delta^{18}\text{O}$ of the coccoliths (‰ deviations from the PDB standard), and $\delta_W = \delta^{18}\text{O}$ of the water of the growth medium (‰ deviation from SMOW). The equations representing the least squares fit to the combined data of *E. huxleyi* and *G. oceanica* is $y = 3.68 - 0.17T$, ($r = 0.91$), and for *C. leptoporus*, *C. carterae*, and *T. heimii* is $y = 0.09 - 0.16T$, ($r = 0.87$).

isotopes during the secretion of the mineral. Isotopic analyses of both planktonic and benthonic foraminifera¹⁶⁻¹⁹ have shown that their tests may be secreted out of isotopic equilibrium. In addition, the secretion of calcium carbonate in echinoderm tests and spines²⁰ and in parts of corals²¹ has been found to reflect non-equilibrium conditions. Calcification in the coccolithophores is intracellular²² in that coccoliths are produced inside each cell and extruded to the cell surface. Paasche²³ determined that bicarbonate rather than carbonate ions are used as a primary source of carbon in coccolith formation, further demonstrating that the deposition of calcium carbonate by coccolithophores is fundamentally different from any inorganic precipitation process. Therefore, comparison with products of extracellular calcification as described above can be made only with some reservation. The vital effect in the coccolithophores may reflect isotopic discrimination effects by cell membranes, as well as by the incorporation in the calcite of metabolic CO_2 or

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Early Jurassic plesiosaurs from Australia

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Remains of plesiosaurs (aquatic reptiles of the suborder Plesiosauria, order Sauropterygia) are known from Cretaceous sediments in every continent¹, including Antarctica². Jurassic plesiosaurs, by contrast, seem to have had a more restricted distribution, and are virtually unknown outside the Northern Hemisphere. We report here the discovery of two plesiosaur specimens in the Lower Jurassic of the eastern part of the Surat Basin, Queensland. These are the earliest adequate examples of plesiosaurs to be discovered in the Gondwana continents, and they greatly extend the known geographical range of early sauropterygians.

The specimens were collected from the upper part of the Evergreen Formation on Kolane property, about 60 km north-east of Wandoan, south-east Queensland. At Kolane, the Evergreen Formation is represented by ferruginous sandstones, thick seams of concretionary ironstone, and argillaceous oolites; these beds are probably an eastwards extension of the Westgrove Ironstone member, as identified towards the top of the Evergreen Formation in the western parts of the Surat Basin^{3,4}. The upper part of the Evergreen Formation has been dated as Upper Liassic on the evidence of plant microfossil assemblages^{3,4}. As well as plesiosaurs, the Kolane locality has yielded poorly preserved plant remains, thin-shelled lamellibranchs, and an almost complete skeleton of the last-known labyrinthodont amphibian⁵.

The less complete of the two plesiosaur specimens (Queensland Museum catalogue no. F10440) is preserved in sandstone and oolite, and comprises a scattering of fragments apparently derived from a single skeleton. The bones are severely weathered, but include recognizable portions of limbs and girdles, and vertebrae bearing rib attachment scars of distinctive plesiosaurian type (Fig. 1a). The specimen is certainly a plesiosaur, but it is so incomplete that its more immediate relationships cannot be determined.

The second specimen (QM F10441) was found about 400 m north of the first, and about 10 m above the site of the Kolane labyrinthodont. It is preserved in blocks of concretionary ironstone, so that its development may present difficulties. This specimen seems to be part of an articulated skeleton, and includes vertebrae, ribs, girdle bones, limb bones and, possibly, gastralia; no cranial elements have been identified with certainty. Two of the cervical vertebrae are exposed on a broken surface and show diagnostic plesiosaurian features allowing identification down to family level (Fig. 1b). Such identification is admissible because detailed studies of vertebrae form the basis of plesiosaur classification^{1,6}. Each cervical centrum is roughly cylindrical, with a fairly flat ventral surface and weakly depressed terminal faces. The floor of the neural canal forms a shallow groove in the dorsal surface of the centrum, so that the latter is slightly kidney-shaped in cross-section. On the ventral surface the large nutrient foramina are separated by a flat area. The standard height/length and width/length indices (ratios $\times 100$) for these cervical centra are about 95 and 115, respectively. Other vertebrae show that the rear margin of each neural spine is vertically grooved—a distinctive feature noted by Welles⁶ in elasmosaurid plesiosaurs, and illustrated by Owen⁷ in plesiosaur vertebrae from the English Lias. A damaged cervical rib seems

originally to have been of characteristic 'hatchet shape'. Structure and proportions of these cervical vertebrae clearly indicate that specimen F10441 is a dolichodiran (or long-necked) plesiosaur, almost certainly referable to the family Plesiosauridae (part of the superfamily Plesiosauroidae).

There are few records of plesiosaurs from Jurassic or earlier rocks of Gondwanaland. The report of a 'suspected plesiosaur' in the Upper Triassic of New Zealand⁸ is founded on a single fragment of limb bone; this fragment could equally well have come from some other type of reptile, or even from an amphibian (see account by W. E. Swinton in ref. 8). A single vertebra from the Upper Liassic of Argentina was originally attributed to a crocodile⁹, but was identified by von Huene¹⁰ as plesiosaurian; Welles considered⁶ that this specimen was not diagnostic. Other records of plesiosaurs from the Jurassic of Argentina^{11,12} are erroneous or founded on indeterminate material^{1,6,13}. Isolated skull fragments of plesiosaurs (plesiosaur superfamily Plesiosauroidae) are known from the Upper Jurassic of India¹⁴ and Ethiopia¹⁵. Fragmentary plesiosaur material was also reported by Bartholomai¹⁶ from an early Cretaceous or late Jurassic conglomerate at Mount Morgan, Queensland.

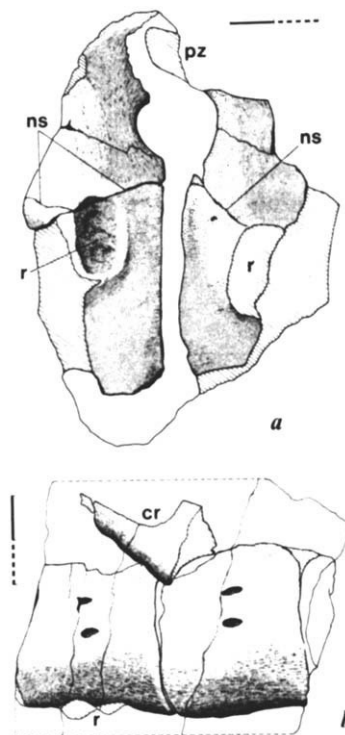


Fig. 1 Plesiosaur vertebrae from the Evergreen Formation (Upper Liassic) of south-east Queensland. *a*, Two anterior caudal vertebrae (from specimen QM F10440) in left lateral view, showing neuro-central sutures (ns), crater-like scars for rib attachment (r) and prezygapophysis of second vertebra (pz). *b*, Two cervical vertebrae (from specimen QM F10441) in ventral view, showing large nutrient foramina, detached cervical rib (cr), and damaged area of rib attachment (r). Matrix is unshaded, and badly damaged surfaces are indicated by oblique shading. Both scales represent 2 cm.

The Kolane specimens are, thus, the earliest adequate examples of plesiosaurs from Gondwanaland. Their discovery greatly extends the known geographical range of early sauropterygians and implies that the Plesiosauria might have had an almost world-wide distribution as early as the Lower Jurassic. The association of the Kolane plesiosaurs with a labyrinthodont amphibian is, at the least, surprising: plesiosaurs typically occur in marine faunas of the Jurassic and Cretaceous, whereas labyrinthodonts are mainly known from continental faunas of the late Palaeozoic and Triassic. The occurrence of a plesiosaur

referable to the Family Plesiosauridae is consistent with the Lower Jurassic age of the Evergreen Formation, and tends to confirm that the labyrinthodont is a late survivor⁵. Reiser and Williams concluded⁴ that most of the Evergreen Formation was laid down in continental conditions, but that the peculiar lithology of the Westgrove Ironstone member might be suggestive of a marine origin. Either the Kolane plesiosaurs were sea-dwellers, and the associated labyrinthodont was accidentally introduced into a marine environment, or the plesiosaurs were non-marine types, ecologically comparable with those, for example, of the English Wealden.

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Kin preference in infant *Macaca nemestrina*

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The ability to recognize conspecifics is a prerequisite for many types of social behaviour, including, for example, parent-offspring relations^{1,2}, mate selection and recognition^{3,4}, territorial defence^{5,6} and dominance coalitions⁷. This ability is of special importance to Hamilton's kin selection hypothesis⁸, which predicts that an individual's behaviour towards a conspecific will depend on the degree of genetic relatedness between them. Although recognition depends on previous experience between individuals in some species^{1,2}, this does not preclude the possibility that recognition could occur in its absence¹². For example, juveniles who disperse before non-littermate siblings are born or adult males who do not participate in rearing their young might benefit from recognition abilities that are independent of prior association between the individuals. Here we show that young pigtail macaques prefer to interact with a related over an unrelated monkey in a laboratory test. Because subjects were separated from their dams at birth and reared apart from all other relatives, results suggest that kin recognition can occur in the absence of prior association with relatives.

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Subjects were 16 infant pigtail macaques (*Macaca nemestrina*) of known genealogies from a captive breeding colony (Table 1). Taken from their dams within 5 min of birth, subjects were placed in an incubator until they became homeothermic and were then transferred to a colony room (7.6 m × 11.0 m) and housed individually in wire cages. Subjects were placed in this room housing 50 other monkeys such that they had no opportunity for visual, physical or olfactory interactions with relatives or any other animals used in the experiments. To ensure normal social development, subjects interacted daily in 'play groups' with four to six similarly aged monkeys, none of whom was related to the subjects.

The Sackett self-selection circus (Fig. 1) was used to measure preference in a free-choice situation. Thus, subjects could select among three simultaneously presented stimuli by first visually orientating towards each one, and later entering the compartment to which a stimulus was attached, orientation time and entry time serving as two measures of preference. Orientation and entry time were recorded by an observer who viewed subjects from a separate room on a closed-circuit television and who knew nothing of the monkeys' genetic relationships.

Subjects were tested once in two different experiments, each using an identical testing procedure but with a different set of choice stimuli. Experiment I offered choices among a subject's paternal half-sibling, an unrelated (to both the subject and other stimulus animal) monkey, and an empty cage to examine our hypothesis that subjects would prefer their relative. Stimulus animals were matched by sex, age, weight, and general activity level and appearance (as subjectively judged by us). Both the subject's half-sibling and unrelated conspecific were delivered and reared identically to the subject. Experiment II examined the strength of the preference predicted in experiment I by providing choices among the subject's paternal half-sibling from experiment I, a 'preferred nonrelative', and an empty cage. The

Table 1 Subjects and their preference times for half-siblings, nonrelatives and an empty cage in experiment I

	Sex	Age (days)	Half-sib stimulus		Non-kin stimulus		Kin entry (s)	Non-kin entry (s)	Empty cage entry (s)	Kin orientation (s)	Non-kin orientation (s)	Empty cage orientation (s)
			Age (days)	Sex	Age (days)	Sex						
BS	M	344	37	F	49	F	189.1	142.3	58.7	78.6	81.2	82.4
BT	F	326	7	M	11	M	174.4	155.5	121.1	88.6	70.2	88.3
CB	M	317	7	M	11	M	400.3	28.1	124.4	87.3	82.1	83.7
DD	F	316	16	M	6	M	226.1	96.3	236.1	169.0	64.4	53.5
DW	M	276	37	F	58	F	390.4	50.7	22.5	178.8	55.6	36.4
LZ	M	206	147	F	145	F	0.8	0.0	599.2	247.9	42.3	4.2
NK	F	159	206	M	193	M	448.3	95.6	0.0	138.7	73.6	74.3
OC	M	149	159	F	160	F	216.8	84.2	66.3	133.4	97.4	37.7
OD	F	147	158	F	159	F	272.9	222.8	8.0	65.6	143.6	69.5
IV	F	91	37	M	30	M	70.2	44.9	60.5	96.5	70.3	94.5
IZ	M	86	20	M	29	M	267.3	192.8	16.2	121.5	62.6	88.6
LC	M	83	20	F	19	F	99.3	82.2	151.8	121.6	55.7	109.2
KL	F	44	92	M	85	M	46.4	0.0	9.0	90.2	70.7	89.9
LF	F	85	24	F	23	F	0.0	488.3	55.5	67.9	107.6	107.7
KK	F	61	17	M	27	M	160.6	222.2	19.4	64.0	127.3	76.8
KP	M	98	10	M	6	M	37.1	257.3	68.7	87.1	122.3	62.0

Orientation time was scored during the first 5 min and entry time during the following 10 min. See text for further details.

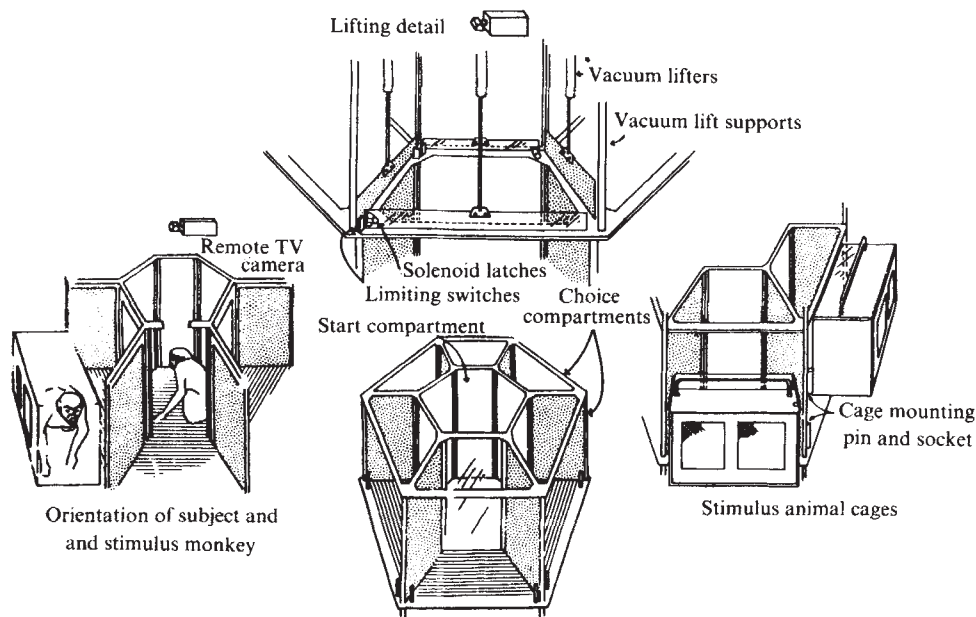


Fig. 1 The Sackett self-selection circus, used in several other studies to measure social preference in primates⁹, was constructed of movable, transparent Plexiglass panels separating the apparatus into a central start compartment and six choice compartments, three of which were used. Plexiglass-carrying cages, each holding the appropriate stimulus, were attached randomly to non-adjacent choice compartments before a subject was placed in the start compartment. Subjects were adapted to the circus before testing. Each subject's preference for the three stimuli was measured during a 15-min test. During the 5-min orientation period when the subject was confined to the start compartment, its visual orientation times, scored whenever its head and body were orientated towards choice stimuli, were recorded by a trained observer (S.R.M.). The observer viewed subjects from a separate room on a closed-circuit television and knew nothing of the monkey's genetic relationships. The start compartment panels were then raised by remote control, and the subject allowed 10 min of unlimited access to the three choice compartments (and start compartment), where it could interact in all ways with the stimulus monkeys except by making physical contact. Entry time elicited by each stimulus was recorded and defined as the total time the subject spent in a particular choice compartment. Subjects typically entered each compartment more than once and also spent time in the start compartment.

preferred nonrelative was obtained for each subject in a separate choice experiment in which two matched stimulus animals, both unrelated to the subject, were presented as choice stimuli. The animal eliciting the greatest amount of entry time was defined as the preferred nonrelative.

Subjects' performances and relevant variables are summarized in Table 1 and Fig. 2. In experiment I, a significant number of subjects (13 of 16) exhibited more entry time toward half-siblings than toward unrelated animals ($P \leq 0.01$, binomial test) and a significantly greater amount of entry time towards half-siblings than towards unrelated animals ($P \leq 0.05$, Wilcoxon signed-ranks test) (Fig. 2a). Subjects' orientation time towards half-siblings was also greater than towards unrelated animals, but this difference was not significant ($P \leq 0.1$). There was no significant correlation between subjects' age and relative kin entry time (kin/total entry time), a measure that controls for developmental differences in mobility. In experiment II, half-siblings elicited more entry time and more orientation time than unrelated animals, although neither of these differences were significant ($P < 0.1$ and $P \leq 0.1$, respectively) (Fig. 2b).

Based on orientation and approach toward half-siblings in experiment I, we conclude that infant pigtail macaques prefer to interact with their half-siblings rather than nonrelatives and, therefore, can discriminate between them. Furthermore, based on rearing conditions of both subjects and stimulus animals, we conclude that this preference and discrimination ability require no previous experience with relatives. Because half-siblings were paternally related, common prenatal experience cannot explain preferences. Of course, each subject experienced itself during rearing which, in some sense, could be considered experience with a relative. And experience with one's own phenotype has been suggested as the basis for demonstrated social preferences in domestic chicks reared in isolation¹⁰.

The data provide no evidence for the proximal basis of the discrimination ability, but results from visual orientation time imply something about the significance of visual cues. Thus, 14 of 16 stimulus animals eliciting the greatest amount of orientation time in experiment I were also favoured in entry time, suggesting that subjects had established preferences even before they were allowed into choice compartments.

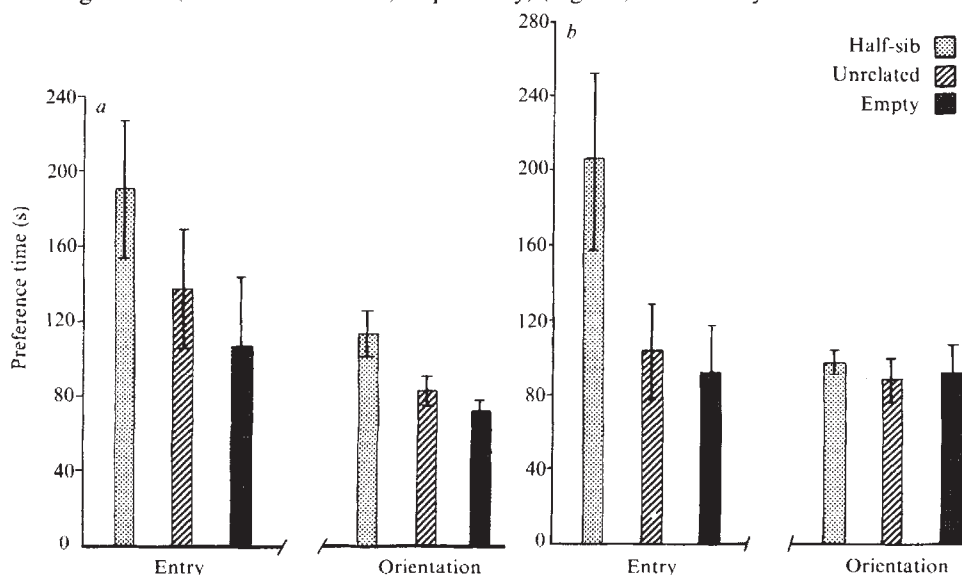


Fig. 2 Mean entry and orientation time ($\pm$ s.e.) elicited by various choice stimuli in experiment I (a) and experiment II (b) ($n = 16$ for each bar). Orientation and entry times were used as two measures of subjects' preference for the choice stimuli during the initial 5-min and the following 10-min periods, respectively. Statistical comparisons (see text) were made with the Wilcoxon signed-ranks test; 1-tailed tests were used based on predicted preferences for half-siblings.

Hamilton argued that selection favours individuals assisting others in proportion to genes shared in common, other things being equal. Although a laboratory study¹¹ has shown that aiding is directed along lines of genetic relatedness among pigtailed reared together, our data are clearly insufficient to demonstrate kin selection. Furthermore, we do not suggest that social preferences would be unaffected by experience; pigtailed do prefer a familiar conspecific to an unfamiliar one⁹. Finally, although our results are based on a single age-class in one species, they do heed us to examine the frequent assumption that recognition of kin requires prior association among relatives.

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Stimulation of the cerebral cortex in the intact human subject

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One of the most fertile methods of investigating the brain is to stimulate a part of it electrically and observe the results. So far, however, use of the method in man has been restricted by the necessity of opening the skull surgically to apply the electrodes. Much could be done, both with healthy subjects and with neurological patients, if it were feasible to stimulate through electrodes on the scalp, although the localization of the stimulus on the cortex will always be much less sharp than with electrodes on the brain surface. In an intact man, however, the brain is protected from electricity by the skull and by the scalp, both of which normally offer considerable resistance. Furthermore, the cerebral cortex does not have a particularly low electrical threshold. It is probably for these reasons (despite an occasional contrary claim¹) that attempts to stimulate the brain by applying stimuli from conventional stimulators to the scalp have been stopped by pain or have otherwise failed. These obstacles have now begun to yield. Recently, it was found that, on stimulating muscles in the human hand² without any special preparation of the skin, the effective resistance fell to low values if brief but very high voltage shocks were used. Applying the same technique to the head, it has now proved possible at the first attempt to stimulate two areas of the human cortex, without undue discomfort.

The stimulating electrodes were ordinary stick-on silver-cup electroencephalogram electrodes of 1 cm diameter, filled with electrode jelly. For the motor area, one electrode was applied initially over the surface marking of the arm area of the motor cortex and the other 4 cm in front. To stimulate, a 0.1- μ F condenser charged to up to $\sim 2,000$ V was discharged through the electrodes using a Morse key. The electrode over the motor area was the positive. A shunt resistance of 100 Ω ensured that the time constant of discharge was less than 10 μ s.

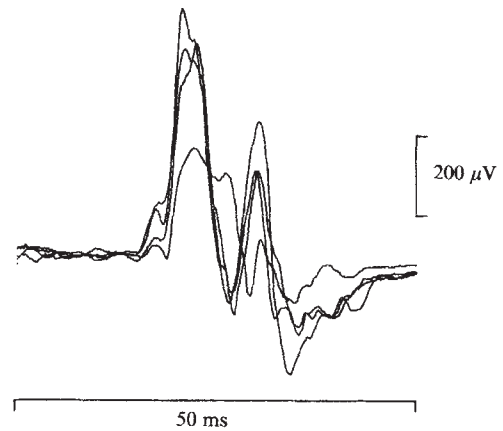


Fig. 1 Stimulation of the arm area of the motor cortex. The records shown are of action potentials from the contracting muscles in the forearm. Stimulation is at the start of the sweep. Four records are superimposed. The latency of responses was 16 ms. (Subject P.A.M.)

Stimulation showed itself by twitch-like movements of the opposite middle and index fingers, or, with the active electrode over the leg area, of the foot. In one subject (height 1.88 m), muscle action potentials recorded through surface electrodes over the active muscles showed fairly sharp latencies of 16 ms for the forearm muscles (Fig. 1) and 34 ms for the muscles in the lower leg. These values agree with those obtained by stimulating the exposed human motor cortex or the nerve fibres leaving it^{3,4}.

With electrodes on the back of the head, over the visual area of the cortex, illusory visual sensations ('phosphenes') were experienced. Larger voltages were necessary for the visual area than for the motor area. For each stimulus the phosphene was very brief. It appeared near the centre of the visual field as a patch, with indefinite edges, subtending some 5°, containing one or a few, sharp, bright sinuous lines. The main evidence that such phosphenes are caused by stimulation of the visual cortex is that they only occurred with electrodes over the visual area and that, within that region, the position of a phosphene in the visual field moved with the position of the stimulating electrodes in a manner that conformed with the known mapping of the visual field on the cortex (half-fields reversed and upside down, with a large area for the centre of the field on the occipital poles). Thus, with a horizontal pair of electrodes above the occipital pole (6 cm above theinion), the phosphene was below the fixation point. It moved upwards roughly to the fixation point when the electrodes were moved downwards (to 3 cm above theinion). Similarly, the phosphenes appeared mainly on the right with electrodes to the left of the mid-line, and vice versa. They disappeared altogether when the electrodes were moved away more than a few centimetres from the occiput.

Another important observation is that the phosphenes described did not disappear when the eyeballs were pressed on until sight was lost in both eyes, so they were not due to the excitation of the retina by spread of current. Such excitation occurs very readily, as the retina has a low electrical threshold; but the resulting phosphenes fill diffusely much of the visual field, are without structure, are not specially related to stimulation over the visual area, and disappear with pressure-blinding. Thus, although both may be seen simultaneously, phosphenes from current spread to the retina are readily distinguished from the phosphenes we attribute to stimulation of the visual cortex.

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Slow current systems in the A-V node of the rabbit heart

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If the heart is to function as an efficient pump, there must be an adequate time delay between the contraction of the atria and that of the ventricles. A major part of this delay is provided by the time of conduction of excitation across the atrioventricular (A-V) node, which electrically links the ventricles with the atria. When the primary pacemaker in the sinoatrial (S-A) node fails to control the cardiac rhythm, as the result of either a depressed automaticity or an impaired conduction, the A-V node can become the pacemaker of the heart¹. In spite of these essential physiological functions of the A-V node, the precise ionic bases of conduction and the automaticity in the A-V node tissue remain unclear. Here we demonstrate the slow inward current as well as the delayed potassium current in the A-V node using the two-microelectrode voltage-clamp technique.

Recently, DeMello² reported the space constant of the rabbit A-V node as 0.4 mm. This clearly indicates that voltage-clamp studies on this tissue are possible using specimens smaller than 0.2 mm. In the present study, small A-V node specimens from the rabbit heart were prepared in essentially the same manner as for the S-A node³. The A-V node was identified by its anatomical localization and the action potential configuration. Small strands of tissue 0.2 mm wide and 1–2 mm long were dissected from the A-V node in a 36 °C Tyrode solution, with their longitudinal axis roughly perpendicular to the fibrous A-V ring. This thin strand was then ligated at two sites with a fine silk fibre (prepared by unwinding commercially available silk suture), to give a final dimension of 0.2 × 0.2 × 0.1 mm.

Most of these small A-V nodal preparations spontaneously discharged action potentials (Fig. 1a) which were quite similar to those reported previously^{4–6}. Voltage homogeneity within this small specimen was tested by using three microelectrodes. Action potentials simultaneously recorded from three different sites (50 µm apart) were closely similar (Fig. 1a). During the voltage clamp, the membrane potential monitored outside the feedback circuit deviated only 5 mV for about 10 ms after the onset of the test pulse (Fig. 1b, c). Therefore, no serious deviation from the command pulse occurred. Similarly good results were obtained in one out of every five specimens tested with three microelectrodes.

Figure 2 shows a family of voltage-clamp records and the current-voltage relationships. The holding potential was -40 mV, where the steady net current was about 0. The transient inward current was recorded between -40 mV and +35 mV, with its peak amplitude at around -10 mV. At -10 mV, the peak of this inward current occurred 5–10 ms after the onset of the test pulse, and the current was inactivated with a time constant of 15–30 ms (Figs 1b, 3a). This slow time course of the transient inward current is quite similar to that of the slow inward current in the S-A node⁷. To identify this current, we superfused this specimen with compound D600 (Fig. 3a), which abolishes the slow inward current. The transient inward current progressively decreased and finally disappeared after 3 min. In contrast, tetrodotoxin, the fast Na⁺ channel blocker, caused no appreciable change in the transient inward current. These

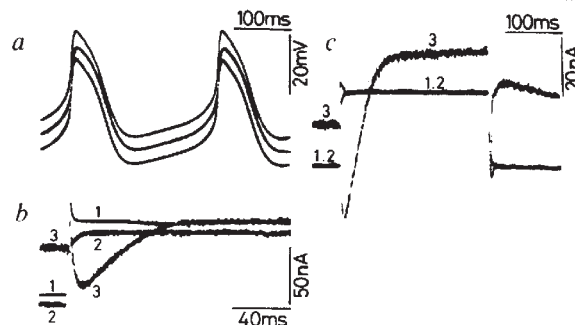


Fig. 1 Voltage homogeneity in the small A-V node specimen tested by three different microelectrodes. The current feeding electrode was positioned at the centre of the endocardial side and other two recording microelectrodes were applied about 50 µm from the edge of the preparation. *a*, Simultaneous action potential discharge at three different sites. Action potential measurements are: overshoot 21 mV, maximum diastolic potential -60 mV, duration of the action potential at 50% repolarization 68 ms, maximum rate of rise 10 V s⁻¹, firing frequency 196 min⁻¹. *b*, Evaluation of spatial clamp during the slow inward current by monitoring the potential outside the clamp circuit (lowest voltage trace). Holding potential -40 mV, test pulse -10 mV. 1, command pulse; 2, monitor potential; 3, current trace. *c*, Spatial clamp during the delayed current. Two potential traces were displayed at the same position on the scope. 1–3, As in *b*. Note the difference in calibration between *b* and *c*.

observations agree well with previous findings that the A-V node action potential was blocked by Ca-antagonists but not by tetrodotoxin^{4–6,8}. Based on these findings, the upstroke of the action potential in our small A-V node preparation is considered to depend predominantly on the slow inward current. Action potentials in the S-A node were shown to propagate at a speed of 2–3 cm s⁻¹ (ref. 9), and a similar conduction velocity was reported for the A-V node¹⁰. Dependency of action potentials in these two structures on the slow inward current would readily explain such a slow conduction.

Delayed current systems in these small A-V nodal specimens were also similar to those found in the S-A node. Depolarization

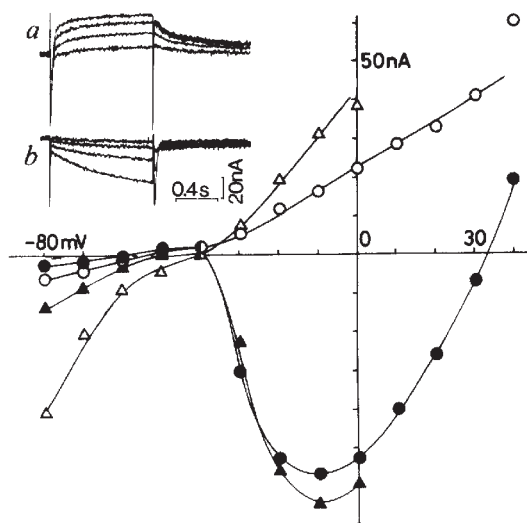


Fig. 2 Current and voltage relationships in the A-V node. Closed symbols indicate transient current, open symbols show the current at 1 s after the change in the potential. Two different experiments are shown. Inset *a* (depolarization) and *b* (hyperpolarization) represent a family of voltage-clamp records in a specimen, from which the curves with triangles were obtained. Duration of the clamp pulse is 1 s.

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caused a slow increase in the outward current, whereas on repolarization, a gradual decay in this current was observed (Fig. 2). The time course of this outward current tail within 1 s was well fitted by a single exponential curve, with a time constant of 189 ± 24 ms (mean $\pm$ s.d., $n = 6$). The reversal potential of the outward current was measured by recording the outward current tail at various potential levels. At 10 mM K^+ , the outward current tail changed its sign at around -60 mV (Fig. 3b),

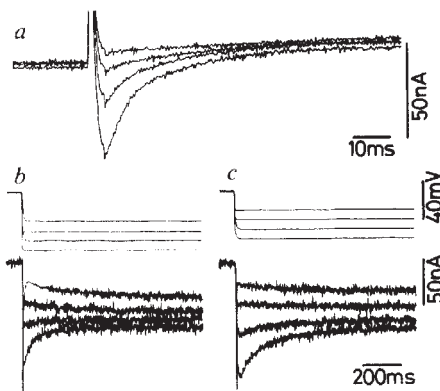


Fig. 3 a, The slow inward current and its progressive decrease induced by perfusion of D600. Current traces from bottom to top were obtained in the control solution, and 1, 2 and 3 min after perfusion of 5×10^{-7} g ml $^{-1}$ D600, respectively. b, c. Reversal potentials of the outward current tail at 10 mM (b) and 20 mM (c) extracellular K^+ concentrations. Top, membrane potential; bottom, current trace. The membrane was depolarized to -10 mV for 1 s and then repolarized to -40 , -50 , -60 , and -70 mV in b and to -30 , -40 , -50 and -60 mV in c. The time constant of the tail current was 100–240 ms at -50 to -70 mV. At the control concentration of K^+ (3 mM) the reversal of the tail current could not be recorded because of a large inward-going rectification.

whereas at 20 mM K^+ , it did so between -40 and -50 mV (Fig. 3c). From the voltage relationship of the outward current tail, the reversal potential was determined as -62 mV and -43 mV at 10 mM and 20 mM K^+ , respectively. Approximately the same slope of 61 mV per 10-fold increase in K^+ concentration was also obtained in three other experiments. These results suggest that K^+ ion is responsible for this outward current. The inward-going current change noted on hyperpolarization from -40 mV to -60 mV (Fig. 2) may be attributed to deactivation of the K^+ current. On the other hand, large inward current changes recorded with further hyperpolarization may be analogous to the current induced by a strong hyperpolarization in the S-A node¹¹ and in the frog sinus venosus¹². It is possible that these delayed current systems are involved in the pacemaker depolarization, as is the case for the S-A node.

The present voltage-clamp study showed that the A-V nodal cells possess transmembrane current systems quite similar to those in the S-A node. A common ionic mechanism may underlie automaticity and slow conduction in these two small but vitally important nodal structures in the heart.

α_2 -Adrenergic receptors appear in rat salivary glands after reserpine treatment

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The regulation of central and peripheral adrenergic receptors by various chemical, physiological, pharmacological and pathological stimuli has been the subject of intense study^{1,2}. For example, drug treatments can produce relatively small changes in the density of existing receptor binding sites in a variety of tissues. The α -adrenergic receptors in rat salivary gland tissue have been studied using radioligand receptor binding techniques³. We have recently identified and characterised α_1 -adrenergic receptors in the rat submandibular gland⁴, but surprisingly, α_2 -adrenergic receptor binding was not detectable. We now report that a single treatment of reserpine results in the appearance of α_2 -adrenergic binding sites within 12 h. Continued treatment with the drug produces further increases in the number of α_2 -adrenergic receptors, such that after 7 days the levels of α_1 - and α_2 -adrenergic receptors are similar. This is the first example of a drug treatment resulting in the appearance of a receptor type which was not previously detectable.

α -Adrenergic receptors, which are heterogeneous in many tissues, have been divided into α_1 - and α_2 -subtypes on the basis of the differential potency of certain drugs⁵. The α_1 -adrenergic receptors are thought to mediate postsynaptic α -adrenergic effects of noradrenaline. In contrast, the α_2 -adrenergic receptors generally appear to be involved in the regulation of pre-synaptic function, and may be localised on the presynaptic membrane. In the appropriate conditions, 3H -clonidine specifically labels α_2 -adrenergic receptors in membrane fractions of various mammalian tissues^{6,7}. Using this radioligand, we have characterised the α_2 -adrenergic receptors in the submandibular gland of control and reserpine-treated rats.

The specific binding of 3H -clonidine to α_2 -adrenergic receptors in glands from control animals cannot be reliably quantified (Fig. 1). However, after seven daily injections of reserpine (0.5 mg per kg, intraperitoneally) the amount of specific binding increased dramatically. Rosenthal analysis⁸ of the data from a saturation experiment indicated a single class of high-affinity receptor binding sites, having a dissociation constant (K_D) of 1.1 nM (Fig. 1). The maximal number of receptors (B_{max}) that could be labelled by 3H -clonidine in these conditions was 6.1 pmol per g wet weight of tissue. In contrast to this large increase in α_2 -adrenergic receptors, α_1 -adrenergic receptors

Table 1 Inhibition of 3H -clonidine binding in submandibular gland membranes from reserpinised rats

Drug	n	K_i (nM)
(-) Adrenaline	7	2.6 ± 0.3
(-) Noradrenaline	3	2.9 ± 0.7
(+) Noradrenaline	1	200
Phentolamine	3	3.6 ± 0.4
Prazosin	5	$5,200 \pm 900$
Yohimbine	5	63 ± 14

IC_{50} values were determined from log-probit analyses of inhibition curves using 10 concentrations of the unlabelled drug in duplicate. The concentration of 3H -clonidine was 0.70 nM (13,000 c.p.m.). K_i values were calculated from the equation $K_i = IC_{50}/(1 + [^3H\text{-clonidine}]/K_D) = 0.73 IC_{50}$.

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increased only 56% (from 5.3 to 8.3 pmol per g tissue) after 7 days of reserpine treatment⁴.

This time course of the appearance of α_2 -adrenergic receptor binding sites is illustrated in Fig. 2. At 12 and 24 h after a single injection of reserpine, the receptor levels were approximately 2 and 3 pmol per g tissue, respectively. When daily injections were continued for 2 to 7 days, the α_2 -adrenergic receptor levels continued to increase, and reached a plateau at a level about double that found after a single injection. Although not presented, an identical type of curve was obtained when the data were calculated in terms of pmol bound per mg of membrane protein. The sites labelled by ^3H -clonidine in glands from animals treated for 7 days with reserpine have the pharmacological specificity and characteristics of α_2 -adrenergic receptors (Table 1). This induction of α_2 -adrenergic receptors is also fully reversible in that 2 days after cessation of chronic reserpine treatment, the levels of α_2 -adrenergic receptors decreased by about 50% and by 5 days approached the undetectable levels in control animals (unpublished data).

A major pharmacological effect of reserpine is the depletion of presynaptic stores of noradrenaline, which in turn, results in a decreased concentration of noradrenaline in the synapse⁹. There is considerable evidence in both central and peripheral systems, that changes in the density of adrenergic receptors are inversely related to changes in the amount of noradrenaline available to interact with these receptors^{1,2}. Thus, it is reason-

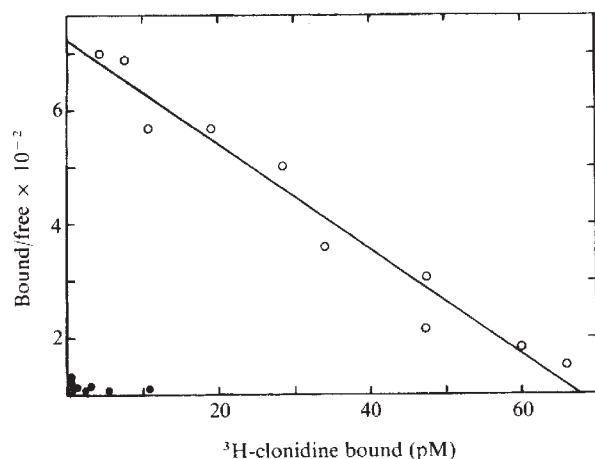


Fig. 1 Rosenthal analysis of ^3H -clonidine binding in rat submandibular gland. Glands were removed from control rats (●), and from treated rats (○) 24 h after the last of seven daily injections of reserpine (0.5 mg per kg, intraperitoneally). The glands were homogenised (Tissumizer) twice in 20 volumes of ice cold 50 mM Tris (pH 8.0 at 25°C) and centrifuged at 49,000g for 10 min following each homogenisation. The pellet (a crude particulate fraction) was suspended in 90 volumes of 50 mM Tris (pH 7.4 at 25°C). For the binding assay, 970 μl of this suspension were incubated for 25 min at 23°C with various concentrations of ^3H -clonidine (22.2 Ci mmol⁻¹, NEN). The suspensions were filtered through GF/B glass-fibre filters (Whatman), washed with buffer, and the radioactivity remaining on the filter was determined. Nonspecific binding, defined by parallel incubation tubes containing 10 μM (–)noradrenaline, was subtracted from the total binding to obtain specific binding. The final concentrations of ^3H -clonidine were from 0.08 to 13.3 nM. For the reserpinised animal, the values calculated from a least squares linear regression analysis are, $K_D = 1.1$ nM, and $B_{\text{max}} = 68$ pM or 6.1 pmol per g tissue or 146 fmol per mg protein. The means $\pm$ s.e.m. for eight experiments are $K_D = 1.9 \pm 0.3$ nM and $B_{\text{max}} = 6.2 \pm 0.9$ pmol per g tissue. A plot of this type is often mistakenly called a Scatchard plot. The Scatchard derivation¹³ assumes a single species of binding macromolecule of known molecular weight and concentration. When these values are not known, as in the present case, where binding is to membrane fragments, it is appropriate to use the Rosenthal plot⁸.

able to propose that the appearance of α_2 -adrenergic receptors in the submandibular gland is a result of the decrease in noradrenaline levels. However, the mechanism of the appearance of these receptors, whether by new synthesis or by the 'unmasking' of existing receptors, is not investigated in our studies.

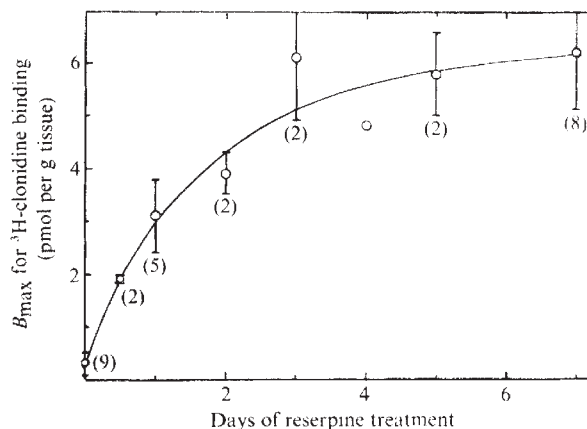


Fig. 2 The effect of the duration of reserpine treatment on the apparent number of α_2 -adrenergic receptor binding sites. Rats were treated daily with reserpine (0.5 mg per kg, intraperitoneally) and then killed 24 h after the last injection (the 0.5 day time point was 12 h after a single injection). The submandibular glands were removed and the B_{max} for ^3H -clonidine binding determined as in Fig. 1. Values plotted are means $\pm$ s.e.m. and the number of experiments is given in parentheses. Although not shown, there were no significant differences in K_D among the various time points. (The mean $\pm$ s.e.m. K_D was 2.05 ± 0.14 nM.)

The reserpinised rat has been proposed as an animal model for the human disease, cystic fibrosis^{10,11}. This hypothesis is the result of studies on rats treated chronically with reserpine which have demonstrated changes in exocrine gland morphology and function similar to alterations observed in patients with cystic fibrosis. Since abnormalities in the autonomic regulation of salivary and other exocrine glands may be a contributory factor in the aetiology of this disease¹², it will be of interest to investigate α_2 -adrenergic function in cystic fibrosis patients.

These data show that the levels of α_2 -adrenergic receptors in the rat submandibular gland can be regulated over a far larger range than has been demonstrated for any other neurotransmitter receptor. This system thus seems ideally suited for the study of the localisation, function and mechanisms of regulation of α_2 -adrenergic receptors.

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Changes of hippocampal Met-enkephalin content after recurrent motor seizures

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Kainic acid (KA), a powerful neurotoxic analogue of glutamate, has been extensively used as a tool for selectively lesioning neuronal cell bodies; however, axons or nerve terminals are spared from damage in the area injected with kainic acid^{1,2}. Injections of this neurotoxin in various brain regions were successfully used to locate cell bodies of neurones containing substance P, enkephalin and other putative neurotransmitters^{3,4}. While attempting to locate the cell bodies of the enkephalin containing neurones present in hippocampus using KA injections, we found that a few days after intracerebral injections of KA a drastic increase in the Met-enkephalin (ME) content of hippocampus occurs. We now describe the delayed increase in hippocampal ME content elicited by intracerebral KA injections and examine the possible mechanism that is operative in causing this increase. Moreover, we provide some evidence suggesting that the increase in ME content elicited by intracerebral injections of KA may be related to the recurrent motor seizures elicited by intracerebral injections of KA.

Male Sprague-Dawley rats (Allison Park) weighing 180–200 g were used. The coordinates for intrahippocampal injection are: A, 3.5; L, 2.4; V, 2.5 according to the atlas of König and Klippel⁵. Unless otherwise stated, rats were anaesthetized with Equithesin (Jensen Salsbury) placed into a stereotaxic holder and injected at constant rate ($1 \mu\text{l min}^{-1}$) with KA dissolved in $1 \mu\text{l}$ of 0.9% NaCl. The concentration of ME in different brain regions was determined by a previously described radioimmunoassay method^{6,7}.

Unilateral intrahippocampal injection of KA ($1 \mu\text{g}$ in $1 \mu\text{l}$ of 0.9% NaCl) caused recurrent motor seizures lasting 4 to 6 h. ME levels in hippocampus and other brain regions were determined at various times after the injection of KA. At 6 to 12 h after the intrahippocampal injection of KA, the ME content in both injected and contralateral hippocampus was slightly lower than before the injection whereas at 24 h after injection the ME content was increased. In the side injected with KA this elevation reached a maximum after 2 to 3 days and lasted more than 2 weeks. In the hippocampus contralateral to the KA

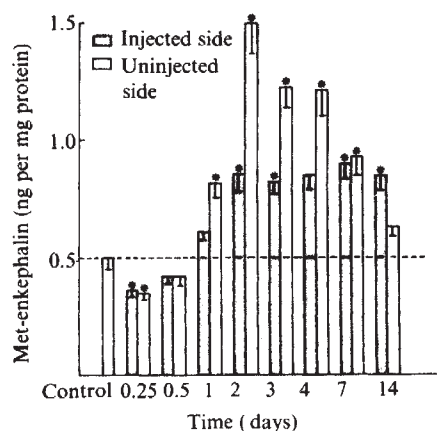


Fig. 1 Time course of change in hippocampal Met-enkephalin content after intrahippocampal injection of kainic acid ($1 \mu\text{g}$ in $1 \mu\text{l}$ of 0.9% NaCl). $n = 6$; * significantly different from control, $P < 0.05$.

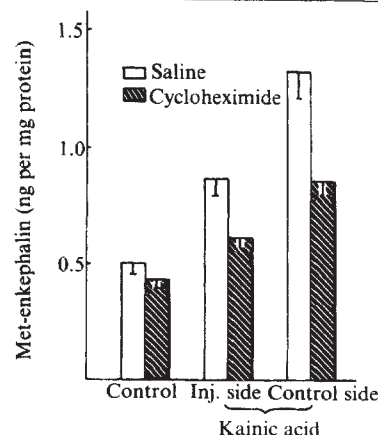


Fig. 2 Hippocampal Met-enkephalin content in control and kainic acid injected rats 24 h after intraventricular injection of cycloheximide or saline. Kainic acid ($1 \mu\text{g}$ in $1 \mu\text{l}$ of 0.9% NaCl) was infused into dorsal hippocampus. At 6 h later the rats received an intraventricular injection of either cycloheximide ($500 \mu\text{g}$ in $20 \mu\text{l}$) or saline through a permanently implanted cannula. Rats were killed 24 h after the administration of cycloheximide or saline. $n = 6$.

injection, the increase in ME content peaked during the first 4 days after the injection and returned to control level by 2 weeks (Fig. 1). A small increase in the ME content (30–40%) was also found in the nucleus caudate and septum; in contrast, the ME content in other brain regions remained unchanged. The levels of substance P, γ -aminobutyric acid (GABA) and glutamate did not increase in either side of the hippocampus 3 days after the intrahippocampal injection of KA, suggesting that the enhancement of ME content elicited by such an injection is a specific effect. A selective increase in hippocampal ME content was also elicited by intraseptal, intrastriatal or intraventricular injections of KA (Table 1). As these injections of KA in various brain regions also caused recurrent motor seizures, it became apparent that the recurrent seizures may be related to the mechanism that triggers the increase in hippocampal ME. This hypothesis was strengthened by the finding that the increase in hippocampal ME content was also observed 2 days after the recurrent seizures induced by either isoniazid or by repeated electroconvulsive shocks (four shocks given at 30 min intervals, data not shown). Moreover, the increase in hippocampal ME content elicited by intrahippocampal KA injection was completely blocked by an injection of phenobarbital which prevents the seizure activity induced by intrahippocampal KA (Fig. 2). Muscimol (like phenobarbital) prevented the increase in hippocampal ME content and the convulsions elicited by KA (data not shown). Thus the evidence we have reported supports the view that recurrent seizures may increase the hippocampal ME content.

Table 1 Hippocampal ME content 3 days after intracerebral injections of KA

Route of injection	Dose	Met-enkephalin (ng per mg protein)	
		Control	Treated
Intrastriatal	($1 \mu\text{g}$)	0.60 ± 0.04	$1.7 \pm 0.1^*$
Intraseptal	($0.5 \mu\text{g}$)	0.50 ± 0.03	$1.5 \pm 0.12^*$
Intraventricular	($0.25 \mu\text{g}$)	0.52 ± 0.03	0.52 ± 0.04
	($0.5 \mu\text{g}$)	0.52 ± 0.03	0.60 ± 0.05
	($1.0 \mu\text{g}$)	0.52 ± 0.03	$1.0 \pm 0.07^*$
	($2.0 \mu\text{g}$)	0.52 ± 0.03	$1.5 \pm 0.12^*$

The coordinates, according to König and Klippel⁵, for the intracerebral injections were: intraseptal (A, 7.8; L, 0; V, 1.0), intrastriatal (A, 7.8; L, 2.5; V, 0) and intraventricular (lateral-ventricle through a permanently implanted plastic cannula). $n = 6-10$.

* $P < 0.05$.

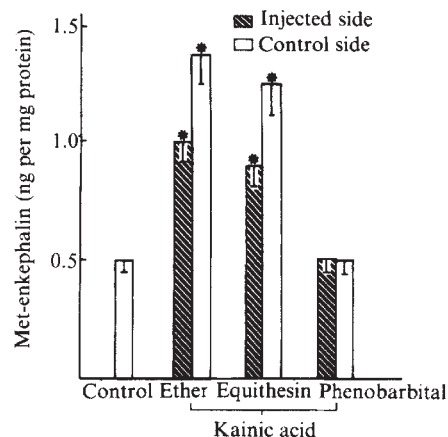


Fig. 3 Effect of pretreatment with phenobarbital (50 mg per kg, intraperitoneally) on the kainic acid induced increase in hippocampal Met-enkephalin content. Kainic acid (1 µg in 1 µl of 0.9% NaCl) was infused into dorsal hippocampus of rats under anaesthesia by either ether or equithesin (main components are chloral hydrate and pentobarbital). For the rats pretreated with phenobarbital, ether was used as anaesthetic. Phenobarbital was given 30 min before and 2 h after the injection of kainic acid. $n = 6$; * significantly different from control value, $P < 0.05$.

The mechanism of metabolic regulation of hippocampal ME is still unknown. The biphasic modification of the hippocampal ME content elicited by intrahippocampal injections of KA indicates that the metabolic state of hippocampal ME can change in response to an increase or a decrease in the rates of neuronal activity. A study of the time course of the elevation of the ME content in the hippocampus ipsilateral or contralateral to the KA injection, revealed that the extent of the increase was greater in the contralateral hippocampus but the increase was longer lasting in the ipsilateral hippocampus (Fig. 1). The persistent modification of the ME content in the injected hippocampus suggests that KA may relieve ME neurones from a tonic inhibition by GABA or other inhibitory neurones.

The hippocampus is important in the modulation of aggression⁸ and memory⁹, and seems to be closely associated with the pathogenesis of epilepsy¹⁰. This region is particularly prone to convulsions¹¹. Stimulation of opioid receptors has an important role in seizures—for instance, microinjection of Leu-enkephalin into the dorsal hippocampus of cats and rats produces epileptiform patterns of electrical activity in the hippocampal EEG. This convulsant action of enkephalin was prevented by microinjection of naloxone¹². Furthermore, naloxone curtails but morphine exacerbates the postictal behavioural depression, suggesting that opioid peptides are released during the seizure and have a role in the postictal depression of motor activity^{13,14}. Although the present study does not make clear the precise mechanism whereby the biphasic change in hippocampal ME content influences seizures, it is clear that the ME content of hippocampus is decreased during recurrent seizures, and these episodes trigger a delayed, long term, rebound increase in the hippocampal ME content.

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Brain noradrenaline depletion prevents ECS-induced enhancement of serotonin- and dopamine-mediated behaviour

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When rats are given a series of electroconvulsive shocks (ECSs) over several days, they display enhanced behavioural responses to both serotonin- and dopamine-receptor agonists^{1–3}. Because these changes are seen when the ECS is given in ways closely mimicking the clinical administration of electroconvulsive therapy (ECT), it has been suggested that the changes in post-synaptic monoamine function may be involved in the antidepressant mechanisms of ECT^{4,5}. Ligand-binding studies have excluded the possibility that ECS alters the characteristics of either the serotonin or dopamine receptor^{6–8}; ECS may therefore be acting on neuronal systems which modulate monoamine neurotransmission. As repeated ECS has recently been reported to decrease both noradrenaline (NA)-sensitive adenylate cyclase⁹ and β -adrenoreceptor binding^{8,10,11}, we have examined here whether changes in NA function are related to the effects of ECS on the serotonin- and dopamine-mediated behaviours. We demonstrate that although selective depletion of NA does not alter the drug-induced serotonin- and dopamine-mediated responses, it abolishes the ECS-induced enhancement of these behaviours.

Rats were anaesthetized with Nembutal (60 mg per kg), and 8 µg of 6-hydroxydopamine (6-OHDA) in 2 µl vehicle was stereotactically injected bilaterally into the locus coeruleus and dorsal and ventral noradrenergic bundles at a level 2 mm caudal to the substantia nigra. The efficacy and specificity of the lesions were confirmed biochemically (Table 1).

Ten days after operation, sham-operated and lesioned rats were given five ECSs (130 V, 50 Hz sinusoidal for 1 s through earclip electrodes) over 10 days (days 1, 3, 5, 8 and 10). ECS-treated rats were compared with animals (sham-operated and lesioned) which had received anaesthetic only. The behavioural responses of the rats to the putative serotonin agonist quipazine (25 mg per kg) or the dopamine agonist apomorphine (2 mg per kg) were examined 24 h after the final ECS or anaesthetic treatment. These behavioural responses were quantified by placing groups of three animals in cages on Automex activity meters (PMS Instruments).

Quipazine induced several abnormal behaviours¹⁴, and hyperactivity components were recorded by the meters. The lesion did not affect the quipazine response in non-shocked animals (Fig. 1). Furthermore, ECS enhanced the quipazine-

Table 1 Effect of the 6-OHDA lesion on whole brain monoamine concentrations

	Brain monoamine concentration (µg per g brain)		
	Noradrenaline	Dopamine	Serotonin
Sham	0.31 ± 0.01 (14)	1.01 ± 0.10 (14)	0.30 ± 0.01 (18)
Lesioned	0.04 ± 0.02 (14)*	0.85 ± 0.09 (15)†	0.31 ± 0.02 (9)

Results show mean ± s.e.m. with number of observations in parentheses. Different from sham-lesioned control values: * $P < 0.001$; † $P < 0.05$. Brain catecholamines were assayed by the method of Chang¹³ and serotonin by the method of Curzon and Green¹⁴.

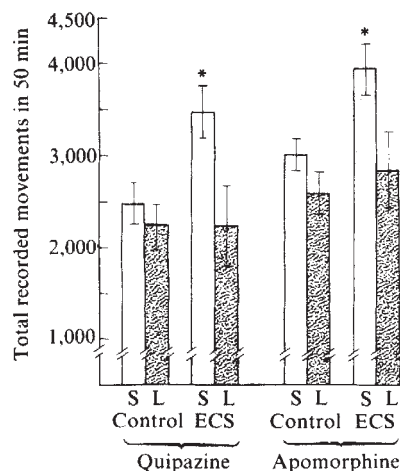


Fig. 1 Effect of 6-OHDA-induced lesions of brain noradrenergic systems on ECS-induced enhancement of quipazine- and apomorphine-mediated locomotor activity. Responses of the sham-operated (marked S and open columns) and noradrenergic-lesioned rats (marked L and stippled columns) are shown. Locomotor activity in groups of three rats following quipazine (25 mg per kg, intraperitoneally) (left hand side) or apomorphine (2 mg per kg, intraperitoneally) (right hand side) was measured in both sham and lesioned groups (control responses). Other groups were given five ECSs spread over 10 days and tested with the two drugs (ECS responses). Results show responses to both drugs in five or six experiments and are reported as the mean $\pm$ s.e.m. of the locomotor counts in the 50 min following drug administration. * Different from appropriate control response of lesioned animals: $P < 0.01$.

induced behaviour in the sham-operated rats, as previously reported in unoperated rats³, but completely failed to alter the hyperactivity response in NA-depleted animals (Fig. 1).

Apomorphine produced a similar locomotor response in both the sham-operated and lesioned rats that had not received ECS (Fig. 1). Following ECS administration there was the expected potentiation of the apomorphine response¹ in the sham-operated animals, but this failed to occur in the lesioned rats (Fig. 1). The degree of enhancement in both serotonin- and dopamine-mediated responses (~30%) produced by ECS administration was very similar to that seen in other recent experiments in this laboratory⁴.

These results demonstrate that destruction of brain noradrenergic systems totally abolishes the ability of ECS to potentiate quipazine- and apomorphine-induced hyperactivity. They also demonstrate that NA is not involved in the mediation of the behavioural syndromes themselves, as the lesion was without effect in non-shocked animals. This result is consistent with the finding that NA depletion with disulfiram did not alter the serotonin-mediated behaviour¹⁵. Our data suggest that changes occur in brain noradrenergic systems during ECS that result in enhancement of serotonin- and dopamine-mediated behaviour. Several observations indicate that ECS does affect noradrenergic systems: repeated ECS increases tyrosine hydroxylase activity¹⁶, accelerates NA turnover^{17,18} and inhibits NA re-uptake at the nerve ending^{19,20}. These observations may indicate an increased release of NA from the nerve ending, which could result in the reduction in β -receptor binding observed in ECS-treated rats^{8,10,11}. However, this interpretation may be challenged by the finding that ECS decreases the sensitivity of adenylate cyclase to NA in rat forebrain slices, as this occurred in 6-OHDA-lesioned rats⁹. Nevertheless, it is clear that presynaptic NA systems must be involved in the production of the enhanced monoaminergic responses we have observed following ECS. This view is supported by our recent finding that catecholamine synthesis inhibition also abolishes the ability of ECS to enhance serotonin- and dopamine-mediated behaviours²².

Although the lesion produced a slight (16%) reduction in brain dopamine content (Table 1), this could not explain our findings, first, because apomorphine is an agonist and second, because the DA denervation would, if anything, increase the apomorphine-mediated response, by producing receptor supersensitivity.

These results demonstrate a noradrenergic mechanism of action of ECS in potentiating serotonin- and dopamine-mediated behaviours. It is tempting to speculate that the antidepressant action of ECS may involve similar mechanisms, in view of the many lines of evidence implicating monoamines in the aetiology of depression and in the mechanism of action of antidepressant drugs (reviewed in ref. 21).

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Partial denervation in inactive muscle affects innervated and denervated fibres equally

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Possible causal factors of denervation-induced changes in muscle include inactivity, products of nerve degeneration and lack of a nerve-borne trophic agent. We now show that if the innervated fibres in a partially denervated rat muscle are rendered inactive, they undergo a reaction as intense as that of the denervated fibres. This provides further support for the view that the effects of denervation on the extrajunctional muscle membrane result from a combination of muscle inactivity and of nerve breakdown products acting diffusely throughout the muscle.

Denervation affects the properties of skeletal muscle fibres through changes which can be attributed in part to inactivity of the muscle^{1,2} and in part to factors arising independently of changes in impulse activity^{3–9}. Thus denervation elicits larger changes in resistance to tetrodotoxin (TTX) of the muscle action potential^{5,8}, fibrillatory activity^{8,9} and extrajunctional acetylcholine (ACh) receptor density^{6,7,9} than muscle paralysis in the absence of nerve degeneration. The difference in efficacy between denervation and paralysis *per se* could arise if cutting the nerve would interrupt the flow of neurotrophic substances to the muscle (for review, see ref. 10). Alternatively, the cut could, by causing nerve degeneration, give rise to pathological factors in the muscle which directly or indirectly affect the muscle fibres.

The latter 'products of nerve degeneration' hypothesis has recently received considerable experimental support^{2,11-13}. For example, in partially denervated muscles not only the denervated but also the remaining innervated fibres develop resistance to TTX, although the innervated fibres respond less strongly¹² (but see refs 14, 15). The weaker response of the innervated fibres could be due to their activity since muscle activity is known to inhibit ACh receptor synthesis¹⁶ and other effects of denervation². In the absence of this suppressive effect

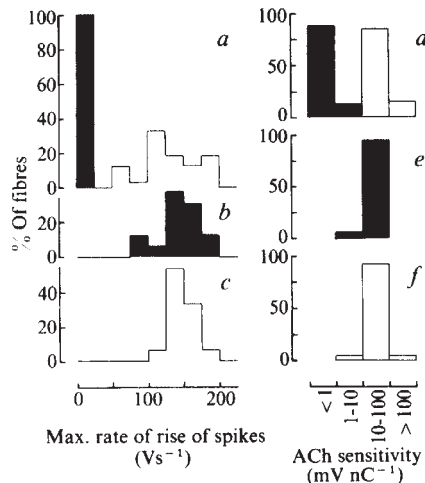


Fig. 1 Resistance of action potentials to tetrodotoxin (a, b, c) and sensitivity to microiontophoretically applied acetylcholine (d, e, f) measured in the same soleus muscle fibres at 1.5–2 mm from the endplate region. Each population is composed of 25–40 fibres from four to five muscles. a And d represent purely impulse-blocked (solid columns) and totally denervated (open columns) muscles. b, c, e And f represent partially denervated muscles (by sectioning the radial nerve L_5) with an impulse-blocked innervation. Innervated (b and e) and denervated (c and f) fibres were distinguished on the basis of presence or absence of miniature endplate potentials (m.e.p.ps) at the endplate region. The percentage of denervated fibres found in the soleus muscles ranged from 36 to 68%. Resistance to TTX was measured at the endplate region, and, in conjunction with sensitivity to ACh, 1.5–2 mm away from it. Two intracellular micropipettes, one for recording (3 M KCl) and the other for passing currents (2 M K citrate) were used, while a third micropipette was put in a just extracellular position for ACh iontophoresis (3 M AChCl). Each fibre was followed over the indicated distance by shifting the recording electrode while applying hyperpolarizing pulses with the current electrode. An alternate pattern of exploration was followed, so that in one fibre the extrajunctional measurements were done first and those at the endplate region second, whereas in the next fibre the reverse order was followed. Resistance to TTX was determined eliciting and recording action potentials in the presence of TTX 10^{-6} M (refs 12, 24). The resting membrane potential was set at -100 mV and the strength of the depolarizing pulse adjusted to give a constant delay of 4 ms between its onset and that of the action potential. The amount of TTX resistance was measured as the maximal rate of rise of the action potential given by its electronically recorded first derivative through an RC circuit ($\times 10^5 \Omega$, 100 pF). Sensitivity to ACh was measured with conventional techniques²⁵. The ACh pipettes were several hundred megohms in resistance and required less than 2 nA braking current. The RMP was set at a constant value of -80 mV with the current electrode while evoking an ACh potential. Units of ACh sensitivity represent millivolts of depolarization per 10^{-9} C of charge passed through the iontophoretic pipette. The oxygenated mammalian Ringer's solution bathing the muscles was kept at a temperature of 28°C . The results obtained for the TTX resistance at the endplate region were similar to those shown in this figure, although all values were moderately higher than in the extrajunctional region, confirming that after denervation TTX resistance develops earlier near the endplates²⁴. Thus, the maximal rate of rise of spikes in the partially denervated muscles was $128 \pm 5.4 \text{ Vs}^{-1}$ ($\pm$ s.e.m.) for the denervated fibres and 127 ± 6.7 for the innervated fibres as compared to 3.1 ± 1.5 for the paralysed control muscles with intact nerves. As far as the EDL muscles are concerned, the results obtained in three impulse-blocked, partially denervated muscles examined between 56 and 72h were similar. For example, the maximal rate of rise in TTX 10^{-6} M at the endplate region was 108 ± 10.3 for 16 denervated fibres and 122 ± 6.9 for 22 innervated fibres, while in the paralysed control muscles with intact nerves the average value was only 11 ± 2.1 (24 fibres). At the time of the acute experiment stimulation of the sciatic nerve proximal to the cuff evoked no contraction of EDL and soleus muscles in partially denervated or control preparations with intact nerves. Stimulation distal to the cuff evoked muscle twitches and transmitted action potentials could be recorded from the muscle fibres before TTX was added to the perfusion fluid. Frequency of m.e.p.ps was normal ($2.3 \pm 0.1 \text{ s}^{-1}$ in 31 innervated fibres of four partially denervated soleus and $2.1 \pm 0.1 \text{ s}^{-1}$ in 29 fibres of three normal soleus).

of activity, the muscle fibres may become particularly sensitive to the effects of degeneration of the nerve. It is therefore possible that these effects may combine with the effects of inactivity to produce the full response to denervation. In that case one would expect innervated but inactive fibres to respond as strongly as adjacent denervated fibres to extensive partial denervation. We have tested this possibility by partially denervating muscles which in addition were paralysed by a chronic conduction block in the nerve.

The extensor digitorum longus (EDL) and the soleus muscles of adult rats were chronically inactivated by blocking impulse conduction in the sciatic nerve with TTX-impregnated cuffs⁶. At the same time the muscles were partially denervated by cutting the radicular nerve L_5 just outside the vertebral canal. Control muscles were either completely denervated or subjected to chronic nerve conduction block only. Soleus and EDL muscles were examined 56–72 h later *in vitro* for the development of extrajunctional sensitivity to ACh and of junctional and extrajunctional resistance to TTX, all essentially absent in normal soleus and EDL muscles^{15,17}. The denervated control muscles were then already highly resistant to TTX and sensitive to ACh (Fig. 1a, d, open columns) while the paralysed control muscles with intact nerves were just beginning to respond (Fig. 1a, d, solid columns). As expected, the denervated fibres in the partially denervated muscles had values comparable to those in the denervated control muscles (Fig. 1c, f). The innervated fibres, on the other hand, were as resistant to TTX and as sensitive to ACh as the denervated fibres (Fig. 1b, e), which was much more than expected from the inactivity alone (Fig. 1a, d, solid columns).

Note that the fibres in the muscles with only about 50% denervation respond as strongly as the fibres in the totally denervated muscles (Fig. 1). This illustrates the efficacy of the effect of partial denervation and one possible explanation would be that at this relatively late time the muscle fibres are already responding maximally. We therefore examined the responses at an earlier time, using muscles in which we had produced different degrees of partial denervation ($\sim 10\%$ to 90%) (Fig. 2). The results show that at 50 h there was a positive correlation between the percentage of denervated fibres and resistance to TTX for both innervated and denervated fibres (Fig. 2). Furthermore, for any degree of partial denervation there was essentially no difference between denervated and innervated fibres. Consequently, in inactive muscles, innervated and denervated fibres give equal responses to partial denerva-

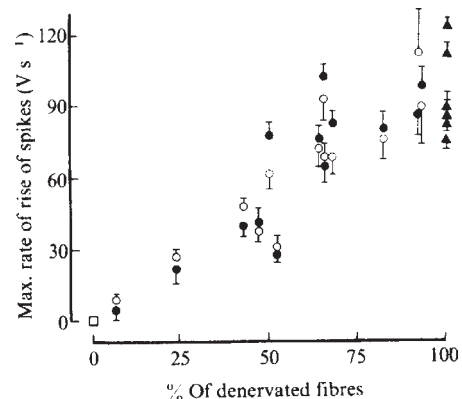


Fig. 2 TTX resistance measured in denervated (●) and innervated impulse-blocked (○) fibres, plotted as a function of the percentage of denervated fibres found in each partially denervated soleus muscle. Each point represents the average TTX resistance measured at 50 h near the endplate region in 4–15 fibres per muscle (the lowest figure applies either to the denervated or the innervated fibres of those muscles exhibiting the lowest or the highest percentage of denervation respectively). Totally denervated (▲) and purely paralysed (□) muscles are also shown for comparison. The lowest percentages of denervation were obtained by incompletely sectioning the radicular nerve L_5 . To obtain a reliable value for the percentage of denervated fibres, 40–50 fibres were recorded in each muscle to observe the presence or absence of m.e.p.ps. Bars represent the standard error of the mean.

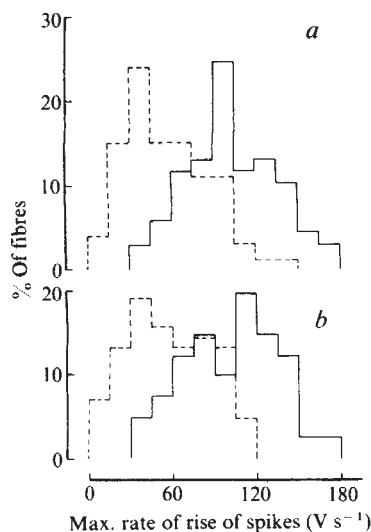


Fig. 3 Class distribution of TTX resistance measured near the endplate region in innervated impulse-blocked fibres (*a*) and denervated fibres (*b*) of 'closely' (columns with continuous lines) and 'distantly' (columns with broken lines) partially denervated soleus muscles at 50 h (about 30 fibres measured per muscle). The percentage of denervated fibres found in the muscles of the two populations was comparable: average 40% for the closely denervated (5 muscles) and 53% for the distantly denervated muscles (8 muscles). The difference in the length of the 'nerve stump' for the two populations was about 6 cm. Although no obvious trauma of the soleus occurred with 'close' partial denervation since the muscle was not exposed the possibility was considered that the greater TTX resistance at 50 h was not a nerve-length effect but a result of muscle damage, particularly because inactive muscles should be more responsive. However, this possibility was excluded because no TTX resistance was observed in the impulse-blocked soleus of three rats in which the attempted close section of the soleus nerve had failed to damage any motor axon.

tion as expected if nerve degeneration causes substances to appear in the interstitium which change the membrane properties of all fibres in that region.

The objection might be raised that nerve degeneration in the periphery is not the only consequence of partial denervation. Axotomy might have central effects which might affect the production of neurotrophic substances in adjacent intact motoneurons. In the frog, for instance, nerve section results in sprouting from contralateral intact axons apparently through changes taking place in the spinal cord¹⁸. To examine this possibility we partially cut the nerve supply either far from the soleus muscle by sectioning (as before) the radicular nerve *L*₅ or close to the muscle by incompletely sectioning the soleus nerve. The innervated fibres were rendered inactive by a TTX-containing cuff around the sciatic nerve. In agreement with previous results obtained in totally denervated muscles⁴, after 50 h the TTX resistance of the denervated fibres was significantly higher in muscles with a short nerve stump than with a long nerve stump (Fig. 3*b*). Identical behaviour was observed in the innervated fibres of the same muscles (Fig. 3*a*), which indicates that the effect on the innervated fibres, as that on the denervated, is peripheral in origin and probably related to the fact that short nerve stumps degenerate more quickly than long nerve stumps¹⁹. Had the effect of partial denervation on the innervated fibres been of central origin, their response would have been expected to lag behind that of the denervated fibres, and to occur earlier in the experiments where the nerve was cut close to the spinal cord.

Thus the present results confirm and extend our previous study¹² by showing that partial denervation also affects the innervated fibres within the muscle. In muscles where the suppressive influence of activity on ACh receptor synthesis and other denervation-like changes^{2,16} has been removed, the responses of innervated and denervated fibres become indistinguishable. This suggests that the response of the muscle fibre membrane to denervation may be accounted for as the combined result of inactivity and the effects of nerve degeneration

per se. The effect could be due to nerve breakdown products acting either directly on the muscle fibres or indirectly via Schwann cells, cells of the immune system or other tissue cells. It should be emphasized that in this work only the effects on the extrajunctional membrane have been studied and that the properties of the postjunctional membrane may be controlled by different mechanisms²⁰⁻²³.

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Proteinase inhibitors suppress the development of experimental allergic encephalomyelitis

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Experimental allergic encephalomyelitis (EAE) is an autoimmune inflammatory disease of the central nervous system (CNS) induced in animals by sensitization with white matter or with myelin basic protein (BP)^{1,2}. EAE is dependent on cell-mediated immune (CMI) reactions requiring T-cell sensitization²⁻⁴, and demyelination is observed in the vicinity of perivascular infiltrates of mononuclear cells⁵. However, the mechanisms by which CMI reactions initiate the clinical and pathological expression of EAE have not been well defined. Numerous mechanisms have been proposed for the degradation of myelin, including activated components of the complement system^{6,7} and the action of enzymes released by inflammatory cells^{8,9}. Among these enzymes, lipases, lysosomal acid proteinases and lymphocyte proteinases have been stressed⁸⁻¹⁴. Recent reports from this laboratory have suggested that damage to the myelin sheath may be initiated by the release of neutral proteinases, including plasminogen activator, from macrophages activated during an immune response¹⁵. If neutral proteinases and, particularly, plasmin, generated from plasminogen by plasminogen activator, have a key role in initiating the clinical signs and pathology of the demyelinating diseases, it might be expected that inhibitors of these enzymes would protect sensitized animals against EAE. Here we have shown that aminomethylcyclohexane carboxylic acid (AMCA), ϵ -amino-caproic acid (EACA) and *p*-nitrophenylguanidinobenzoate (NPGb), which are inhibitors of plasminogen activator and other neutral proteinases, gave significant protection against the clinical expression of EAE in Lewis rats. Other protease inhibitors gave partial or no protection.

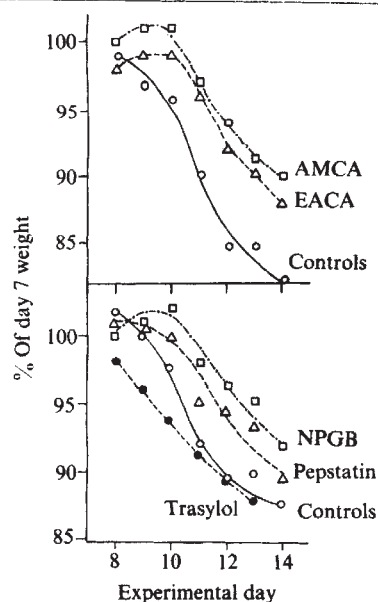


Fig. 1 Weight loss curves for two experiments. Animals were sensitized and drugs administered as described in Table 1 legend. The mean weight for each group is expressed as percent of the mean weight of the respective group at day 7.

Male inbred Lewis rats weighing ~300 g were injected on day 0 in each hind footpad with 0.1 ml of an emulsion containing a freshly prepared 50% suspension in saline of guinea pig spinal cord in an equal volume of complete Freund's adjuvant containing 10 mg ml^{-1} of heat-killed *Mycobacterium tuberculosis*, H 37 RA. Starting on day 6, proteinase inhibitors were injected intraperitoneally twice daily, and sensitized control animals were injected with sterile non-pyrogenic saline. Thereafter, rats were weighed daily and scored for clinical expression of EAE. Control animals consistently became ill on days 12–14. On day 16, animals were anaesthetized with Nembutal, killed and prepared for histology by whole body perfusion with formaldehyde for haematoxylin and eosin staining, or with glutaraldehyde for epon embedding and staining with toluidine blue¹⁶.

Table 1 shows the proteinase targets of the respective inhibitors and summarizes the results for prevention of paralysis and pathology in the sensitized animals. AMCA was the most effective agent; EACA, NPGB and pepstatin consistently protected some of the animals. Leupeptin and antipain, which are proteinase

inhibitors of microbial origin¹⁷, did not prevent paralysis at a dose of 1 mg twice a day, and insufficient material was available for testing at higher doses.

AMCA is 5–10 times more effective than EACA as an inhibitor of fibrinolysis^{18,19}, and consistent with this is the finding that AMCA was more effective than EACA in protecting against EAE (Table 1). However, interpretation of the clinical data for NPGB, pepstatin and Trasylol is more complicated. NPGB, which also inhibits plasminogen activator and plasmin, becomes toxic at approximately 10 mg per day, is difficult to solubilize and hydrolyses readily. We suspect that the beneficial effects of NPGB in inhibiting EAE are offset by toxic effects of the hydrolysis product, *p*-nitrophenol²⁰. Of the inhibitors used, AMCA and NPGB were most effective in decreasing the extent of weight loss which is characteristic of EAE (Fig. 1).

Pepstatin was also difficult to solubilize, and initially it was administered as a suspension in 10% ethanol. In these experiments, six out of nine animals failed to become paralysed. In the experiment shown in Fig. 1, pepstatin protected against weight loss, but this effect was not consistent. To control for the possibility that either the alcohol solvent or the particulate nature of the polypeptide, pepstatin, was protective, we treated rats with a suspension of polyvaline in 10% ethanol. Polyaline, which is not known to inhibit proteinases, did not protect against EAE (Table 1). In animals treated with Trasylol, the onset of clinical signs was earlier and paralysis more severe than in the control animals (Table 1), and the animals lost more weight than the controls (Fig. 1).

The degree of infiltration of the CNS by inflammatory cells was evaluated histologically. Routine haematoxylin and eosin staining showed some pathological evidence of disease in animals from all the groups, but in animals successfully treated with pepstatin and NPGB (Fig. 2), both perivascular infiltration and sub-meningeal inflammation were markedly reduced. In asymptomatic animals treated with the antifibrinolytic drugs AMCA and EACA, haematoxylin and eosin sections showed perivascular infiltration to be reduced only slightly; however, it was obvious from toluidine blue-stained sections of AMCA-treated animals that the extent of invasion into the white matter and the degree of demyelination in the vicinity of the inflammatory cells were reduced considerably (Fig. 3). Histological examination of paralysed animals receiving Trasylol demonstrated a widespread diffuse cellular invasion at all levels of the spinal cord and brain, and the degrees of infiltration and demyelination were more severe than in paralysed control animals.

The findings that Trasylol exacerbated EAE and that pepstatin, an inhibitor of acid proteinases, conferred partial protection against EAE, would seem to be inconsistent with our hypothesis. Two modes of action for pepstatin can be considered. One is that

Table 1 Protection of sensitized rats from EAE by injection of proteinase inhibitors

Drug	Proteinase(s) vulnerable to drug <i>in vitro</i>	Daily dosage	Perivascular infiltration (spinal cord)	Clinical grade	Effect on EAE rats paralysed/total
Controls			2.9	4.0	30/35
AMCA	Neutral proteinases, incl. Plg A and plasmin ^{18,19}	100 mg	2.3	0.46	2/16
EACA	Neutral proteinases, incl. Plg A and plasmin ^{18,19}	1.6 g	2.0	1.8	4/10
NPGB	Plasmin; Plg A; trypsin; thrombin ^{20,34}	2 mg	1.6	1.6	7/22
Pepstatin	Acid proteinases; cathepsin D ¹⁷	2 mg	1.5	1.5	3/9
Leupeptin	Neutral proteinases; cathepsins A and B ¹⁷	2 mg	3.0	3.7	7/8
Antipain	Neutral proteinases; cathepsins A and B ¹⁷	2 mg	2.5	2.2	3/5
Trasylol	Neutral proteinases ^{18,19}	4,000 units	3.2	4.5	9/10*
Polyvaline	None; control for pepstatin	2 mg	3.0	4.0	5/5

EACA, AMCA (both Sigma), leupeptin, antipain and Trasylol (Bayer) were administered in sterile non-pyrogenic saline, NPGB (ICN Nutritional) was administered in 1% dimethyl sulphoxide (DMSO) in saline or in 10 mM sodium acetate buffer, pH 3.6, in saline and pepstatin (Bristol Laboratories) and polyvaline (ICN Nutritional) were administered as suspensions in 10% ethyl alcohol in saline. Beginning on day 7 after sensitization, rats in the experimental groups were injected with inhibitors twice daily, and rats in the control groups were injected with saline twice daily. We also tested pepstatin in 2% DMSO. No protection was observed, but because the control animals tested with 2% DMSO alone showed marked exacerbation of the disease, the results are not included here. Clinical grades on a scale of 1–6 were evaluated by three independent investigators according to the protocol of McFarlin, Blank and Kibler³⁵; and perivascular infiltration was graded on coded slides using an arbitrary scale of 1–4. Abbreviations used: Plg A, plasminogen activator; EAE, experimental allergic encephalomyelitis; AMCA, transaminomethylcyclohexane carboxylic acid; EACA, ϵ -aminocaproic acid; NPGB, *p*-nitrophenylguanidobenzoate.

* Rats became paralysed 2 days before controls.

acid proteinases, which can be secreted by macrophages²¹, may be involved in the clinical and pathological expression of EAE⁹⁻¹⁴. Alternatively, pepstatin in suspension might be toxic for phagocytic cells. Although we cannot rule out either of these possibilities, the histological data for NPGb and pepstatin suggest that these drugs may act at stages in the activation of the cell-mediated immune system before the entry of macrophages into the CNS.

The histological picture for AMCA, where the infiltrating mononuclear cells characteristic of EAE were found but demyelination was not observed (Fig. 3), strongly suggests that AMCA protects against demyelination by inhibiting plasminogen activator, plasmin or other neutral proteinases after macrophage infiltration of the white matter.

AMCA and EACA have had limited clinical application in recent years in treating subarachnoid haemorrhage, angioneurotic oedema and chronic urticaria, with absent or moderate side effects²²⁻²⁹.

Of the series of proteinase inhibitors used in the present studies on EAE, the partial efficacies of pepstatin, NPGb and EACA are consistent with the results of other investigators³⁰⁻³³. The degree of protection obtained with NPGb gives further credence to the role of serine proteinases in EAE, especially if one takes into consideration the toxicity of NPGb. The results obtained with Trasylol (aprotinin) are disturbing and difficult to explain but have been independently observed by Smith³³. It will be possible to speculate on the mechanisms involved when more data become available regarding the effects of Trasylol on circulating and tissue levels of neutral proteinases, particularly plasmin and plasminogen activator. The results suggest caution in the possible use of any of these drugs in humans. The most significant observation is the marked protective effect of the antifibrinolytic agent, AMCA. In animals treated with AMCA the histological evidence of inhibition of demyelination in the vicinity of infiltrating mononuclear cells supports the hypothetical participation of the macrophage-secreted plasminogen activator in the pathogenesis of EAE, and, possibly, in other inflammatory demyelinating conditions.

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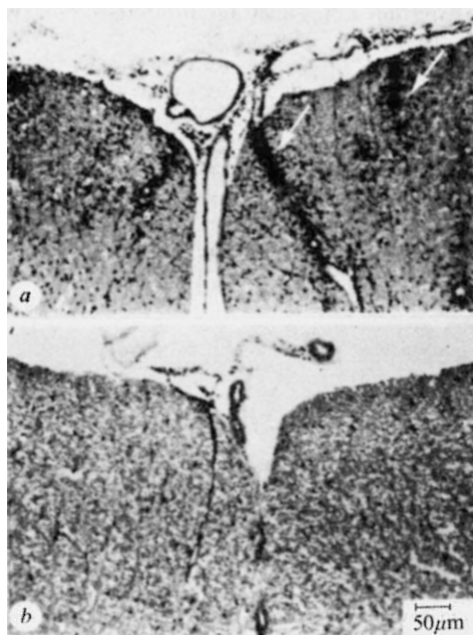


Fig. 2 Sections (7 µm) stained with haematoxylin and eosin. *a*, Paralysed rat injected twice daily with 1 ml of sterile saline. Arrows indicate perivascular cuffs in the anterior columns of the spinal cord. *b*, Asymptomatic rat injected twice daily with 1 mg of NPGb in acetate buffer. Note the absence of sub-meningeal inflammation and perivascular cuffing in the anterior columns of the spinal cord.

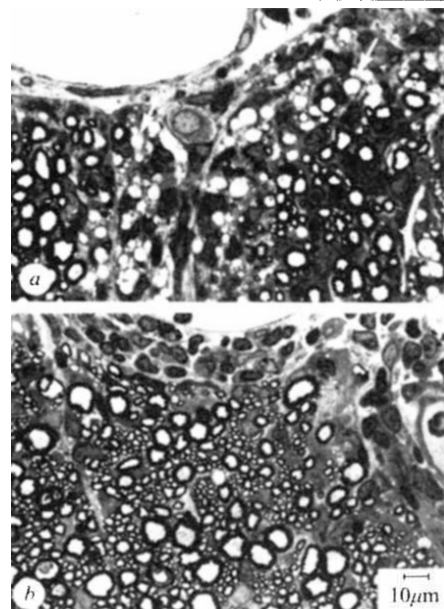


Fig. 3 Toluidine blue (1 µm) stained epon sections of spinal cords from Lewis rats perfused 16 days after sensitization with guinea pig spinal cord in CFA. *a*, Paralysed rat injected twice daily with 1 ml of sterile saline, starting on day 6. Arrows indicate demyelinated axons in the vicinity of the infiltrating cells. *b*, Representative slide of spinal cord from asymptomatic rats injected twice daily with 1 ml AMCA (50 mg ml⁻¹) in saline, starting on day 6. Note the perivascular cuff of mononuclear cells, and the marked absence of demyelinated axons.

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Myasthenia gravis induced by monoclonal antibodies to acetylcholine receptors

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Autoantibodies to nicotinic acetylcholine receptors (AChRs) are demonstrable in sera of approximately 90% of patients with myasthenia gravis (MG), a disease of neuromuscular transmission¹. The consistent abnormality in MG is a low amplitude of miniature endplate potentials (m.e.p.ps) due to decreased sensitivity of the postsynaptic membrane to the neurotransmitter, acetylcholine (ACh). This reflects a decrease in numbers of AChRs in the membrane (for review see ref. 2). The pathogenicity of anti-AChR antibodies has been documented *in vivo* by passive transfer of MG from human to mouse³ and from rat to rat⁴ and *in vitro* on cultured muscle cells⁵⁻⁸. However, titres of anti-AChR antibodies in serum do not correlate closely with severity of muscle weakness in MG patients¹. This lack of correlation very probably reflects the heterogeneity of specificities of anti-AChR antibodies which is known to exist in MG². For example, antibodies directed to determinants of AChRs which are inaccessible *in vivo* may be generated in the course of the disease without being relevant to disease pathogenesis. Identification of antigenic determinants of AChRs which are the primary target(s) of antibody attack in MG is a logical prerequisite to designing antigen-specific immunotherapy for this disease. We report here the production by hybridoma cells of monoclonal rat antibodies reactive with muscle AChR, both adult and fetal, in all of six mammalian species studied. Antibodies produced by two of these hybridoma lines bound to one or more antigenic determinant(s) exposed extracellularly, but remote from the binding site for cholinergic ligands, in the postsynaptic membrane of muscle, and caused a defect of neuromuscular transmission in mice, rats and guinea pigs. These findings establish conclusively that neuromuscular transmission can be impaired by monospecific anti-AChR antibodies which do not inhibit the binding of neurotransmitter to its receptor site.

Cells of a mutant mouse myeloma line, S194/5.XXO.BU.1, resistant to 1×10^{-4} M bromodeoxyuridine (BUdR), and a non-producer of immunoglobulin were maintained in Dulbecco's modification of Eagle's medium supplemented with 10% fetal calf serum; BUdR (1×10^{-4} M) was present for 72 h preceding fusion. AChR-primed lymphocytes were obtained from female Lewis rats inoculated at approximately monthly intervals with

AChR (purified from the electric organ of *Torpedo californica* by affinity chromatography on α -neurotoxin of *Naja naja siamensis* venom) with adjuvants⁹. At 4-5 days after final challenge with AChR, 10^8 lymphocytes from regional lymph nodes were mixed with 10^7 myeloma cells for fusion in 43% (vol/vol) polyethylene glycol 1500 (BDH) as described by Galfre *et al.*¹⁰. Cells were dispensed (2×10^6 lymphocytes per 2.0-ml well) in selective hypoxanthine/ aminopterin/thymidine HAT medium, with 2-mercaptoethanol, 1×10^{-4} M, into multi-well dishes (Costar no. 3524) seeded 1 day previously with rat peritoneal cells¹¹. Hybrid cells producing antibodies of interest were cloned by limiting dilution in micro-well plates (0.4 cells per 0.2-ml well). To ensure monoclonality, each clone selected for study was isolated from a second cloning at this low density. Hybridomas secreting antibodies to AChR were identified by an immunoprecipitation radioimmunoassay⁴ using solubilized AChR complexed with an excess of ¹²⁵I- α -bungarotoxin (α -BT), a cholinergic ligand which binds essentially irreversibly to the receptor's binding site for ACh. Nonspecific binding of α -BT or AChR (c.p.m. precipitated in non-immune serum) was subtracted.

Fusion of a non-secreting mouse myeloma with lymph node cells from rats primed in optimal conditions (5 days after a secondary or tertiary intradermal challenge by AChR) yielded reproducibly a high frequency of hybridomas; in five experiments, hybridoma cells appeared on initial plating in a mean of 73% of the wells; all these produced antibodies reactive with *Torpedo* AChR. In individual fusion experiments 26-100% of the hybridomas recognized determinants of *Torpedo* AChR which were shared by AChR of mammalian skeletal muscle.

The three cloned lines studied in detail here have been passaged continuously for 11-15 months without loss of specific antibody production. Their patterns of reactivity with AChR from a variety of sources are shown in Table 1. McAb-1 recognizes a determinant unique to *Torpedo*; McAb-2 and McAb-3 recognize determinants common to AChR of *Torpedo* and adult and fetal skeletal muscle from all of six mammalian species tested. Variation in the degree of binding to AChR of separate species (and to fetal and adult AChR of dog and cat) suggests differences in conformation, accessibility and perhaps numbers of individual determinants on AChR solubilized in non-ionic detergent from membranes of muscle from different sources. To determine whether a monoclonal antibody might bind to antigenic determinants exposed extracellularly in innervated mammalian muscle *in vivo*, athymic male BALB/c nude (nu/nu) mice (ARS Sprague-Dawley), about 7 weeks old, were injected intraperitoneally with $0.2-1.0 \times 10^8$ living hybridoma cells. Mice injected with hybridomas secreting McAb-2 and McAb-3 showed no signs of weakness, weight loss or tumour in 46 days of observation. However, microelectrode studies of forearm and diaphragm muscles¹², carried out between days 7 and 46, revealed a highly significant ($\approx 50\%$) reduction in

Table 1 Specificities of three monoclonal antibodies: comparison of binding to AChRs of different species

Hybridoma Clone	<i>Torpedo</i> *	Relative AChR binding in % of <i>Torpedo</i> AChR bound									
		Rat		Human		Guinea Pig		Dog		Cat	
		A	F	A	F	A	F	A	F	A	F
McAb-1	100	0	0	0	0	0	0	0	0	0	0
McAb-2	100	57	56	40	38	15	19	34	14	36	13
McAb-3	100	71	69	45	48	24	19	52	22	51	20

Several precautions were taken to ensure valid comparison of binding specificities of the monoclonal antibodies. First, affinity-purified *Torpedo* AChR was used as a reference antigen. Preliminary assay was carried out to ensure that dilutions of antibody selected bound approximately 50% of *Torpedo* AChR (that is, in the linear range of binding). Binding of mammalian AChR was then expressed as per cent of the binding of *Torpedo*. To ensure that in each assay the same constant excess amount of mammalian muscle AChR was added, aliquots of each muscle extract were assayed for their AChR content using an excess of a polyspecific rat anti-AChR antiserum. All assays were carried out simultaneously in duplicate with a single batch of reagents. AChR was extracted in 2% Triton X-100 buffer¹⁵. Only in the case of *Torpedo* was AChR purified (by affinity chromatography on α -neurotoxin agarose). Adult muscle (A) was obtained from limbs of human (amputations), guinea pig, dog and cat, and from eviscerated carcasses of rat and mouse. Fetal muscle (F) was obtained from limbs of human (spontaneous abortions at 23 and 24 weeks gestation), dog (near term), cat (near term) and carcasses of fetal rat (19 d) and guinea pig (8 cm). Although the fetuses selected were at comparable stages of maturation, there is no evidence from species other than rat^{22,23} that antigenic differences exist between forms of AChR found in fetal and mature adult muscle.

* Two factors may account for the consistently greater binding to *Torpedo* AChR by McAb-2 and McAb-3: first because *Torpedo* AChR was purified, its antigenic sites would be more accessible for binding; second, solubilized *Torpedo* AChR exists in a predominantly dimer form²⁴, in contrast to the exclusively monomeric form of mammalian muscle AChR.

Table 2 Pathogenicity of anti-AChR antibodies secreted by clonal hybridomas in nude mice

Cells inoculated	m.e.p.p. amplitude*		Serum antibody† titre ($\times 10^{-9}$ M $\pm$ s.e.m.)	Muscle AChR‡	
	Forearm (mV $\pm$ s.e.m.)	Diaphragm (mV $\pm$ s.e.m.)		Total content ($\times 10^{-11}$ moles per mouse, s.e.m.)	% Complexed with immunoglobulin $\pm$ s.e.m.
Nil	0.79 $\pm$ 0.042 (7)	1.41 $\pm$ 0.107 (7)	0.0 (11)	1.39 $\pm$ 0.060 (9)	0
McAb-1	0.73 $\pm$ 0.083 (6)	—	0.0 (6)	1.47 $\pm$ 0.050 (12)	0
McAb-2	0.38 $\pm$ 0.086 (4)§	0.70 $\pm$ 0.109 (4)§	8.7 $\pm$ 2.77 (4)	0.93 $\pm$ 0.143 (8)	52 $\pm$ 10
McAb-3	0.39 $\pm$ 0.027 (6)§	0.63 $\pm$ 0.086 (6)§	227.4 $\pm$ 130.51 (6)	0.49 $\pm$ 0.032 (6)	86 $\pm$ 4

* Microelectrode studies were done as described elsewhere¹². m.e.p.p. amplitudes were corrected for non-linearity of the endplate response to ACh and to a resting potential of -75 mV. m.e.p.p. with rise time of 0.6 ms or less were measured at 31 °C. Difference in m.e.p.p. amplitude between forearm and diaphragm is related to smaller diameter of muscle fibres in diaphragm. Diaphragms from mice inoculated with McAb-1 cells were not tested because of adherent tumour cells which were not present in McAb-2 and McAb-3. Numbers in parentheses are number of mice.

† Sera were obtained on days 31–46 and 7–27, respectively, after intraperitoneal inoculation with McAb-2 and McAb-3 hybridomas. Mouse muscle AChR, solubilized in Triton X-100 and complexed with an excess of ¹²⁵I- α -BT, was used as antigen¹⁵. Sera from mice bearing McAb-1 hybridoma cells (obtained on days 12–18) had no detectable antibody reactive with mouse muscle AChR, but titres of antibody reactive with *Torpedo* AChR were extremely high (mean $4,000 \pm 289 \times 10^{-9}$ M), reflecting the prolific intra-abdominal growth of the McAb-1 hybridoma. Binding of *Torpedo* AChR by sera of mice bearing McAb-2 and McAb-3 was similar to their binding of mouse muscle AChR (mean titres for *Torpedo* AChR, $12.0 \pm 2.93 \times 10^{-9}$ M and $247.7 \pm 148.05 \times 10^{-9}$ M, respectively).

‡ AChR was extracted in 2% Triton X-100 buffer from homogenized carcasses of individual mice and labelled with an excess of ¹²⁵I- α -BT. Total AChR and % complexed *in situ* with antibody were determined by immunoprecipitation¹⁵. Reductions in AChR content of McAb-2 and McAb-3 carcasses are highly significant ($P < 0.01$ and < 0.001 , respectively).

§ Significantly different from non-inoculated group (Student's *t*-test, $P < 0.001$). Forearm m.e.p.p. amplitudes are also significantly different from those of McAb-1 group ($P = 0.02$ and < 0.01 for McAb-2 and McAb-3 groups, respectively). McAb-1 is not significantly different from control ($P = 0.5$).

Table 3 Pathogenicity of monoclonal anti-AChR antibodies passively transferred into rats and guinea pigs

Immunoglobulin injected*	Incidence of muscle weakness	m.e.p.p. amplitude†		Serum titre of antibody to homologous muscle‡ AChR ($\times 10^{-9}$ M $\pm$ s.e.m.)	Muscle AChR§	
		Forearm (mV $\pm$ s.e.m.)	Diaphragm (mV $\pm$ s.e.m.)		Total carcass ($\times 10^{-11}$ moles $\pm$ s.e.m.)	% Complexed with immunoglobulin $\pm$ s.e.m.
Rats						
Control	0/10	0.63 $\pm$ 0.021 (6)	0.89 $\pm$ 0.059 (4)	0.0	4.22 $\pm$ 0.342 (10)	0
McAb-1	0/2	—	1.05; 0.67	0.0	4.66; 4.10	0
McAb-2	10/10	—	0.36 $\pm$ 0.100 (4)	2.9 $\pm$ 0.27 (8)	1.67 $\pm$ 0.192 (8)	35 $\pm$ 1.4 (8)
McAb-3	8/8	0.10 $\pm$ 0.018 (7)	0.31 $\pm$ 0.067 (4)	11.3 $\pm$ 1.54 (8)	1.23 $\pm$ 0.103 (8)	50 $\pm$ 2.8 (8)
Guinea pigs						
					Forearm (moles per g $\times 10^{-13}$)	
Nil	0/3	0.80 $\pm$ 0.061 (3)	—	0.0	5.68 $\pm$ 0.324 (3)	0
McAb-3	2/2	0.38; 0.25	—	0.21; 0.63	1.56; 1.59	11; 37

* McAb-1, -2 and -3 immunoglobulins were purified from growth medium of hybridoma clones by affinity chromatography on agarose-conjugated goat anti-rat immunoglobulin¹⁸. Female Lewis rats and adult male guinea pigs were injected intravenously with immunoglobulin (1×10^{-10} moles in each rat and 8×10^{-10} moles in each guinea pig) and killed 2–3 days later. Control immunoglobulin was precipitated by 40% saturated ammonium sulphate from pooled serum of rats inoculated with adjuvants only, and 100 μ g was injected into each rat.

† m.e.p.p. amplitudes were measured as described in Table 2 legend. McAb-2 and McAb-3 are significantly different from control ($P < 0.005$ and $P < 0.001$, respectively).

‡ Titre of antibodies in rat and guinea pig serum was determined as described in Table 2 legend but using rat and guinea pig muscle AChR, respectively. Titres of antibodies reactive with *Torpedo* AChR in sera of rats injected with McAb-1 were 4.7 and 8.2×10^{-9} M.

§ AChR content of muscle was measured as described in Table 2 legend. Values for McAb-2 and McAb-3 rats were significantly lower than for the control group ($P < 0.01$ and < 0.001 , respectively).

amplitude of m.e.p.ps in both muscles when compared with the age-matched, non-inoculated mice (Table 2). Furthermore, McAb-2 and McAb-3 antibodies were bound extensively to AChRs of skeletal muscle in the mice and the total amount of AChRs in the muscle was reduced. These three abnormalities are characteristic of MG occurring spontaneously in humans¹³ and dogs¹⁴ and in experimental autoimmune MG (EAMG) induced by active immunization with purified AChRs in rats¹⁵ and in mice¹⁶. Failure of the mice to develop weakness reflects their large safety factor of neuromuscular transmission. Although the reduction in m.e.p.p. amplitude of 50% is highly significant, it is not sufficient to cause clinical signs of weakness in mice or rats¹². The hybridoma line secreting McAb-1 was tumorigenic in nude mice, and sera of those mice had very high titres of antibodies reactive with *Torpedo* AChR (mean = $4,000 \pm 289 \times 10^{-9}$ M). However, the immunoglobulin, which recognized only *Torpedo* AChR *in vitro*, did not bind to muscle AChR in the mice and m.e.p.p. amplitudes did not differ significantly from those of age-matched normal nude mice.

To demonstrate that the hybridomas were producing true autoantibodies, McAb-2 and McAb-3 cells (1.4 and 3.5×10^8 cells) were injected intraperitoneally into 10-week-old female Lewis rats. Within 24 h profound muscle weakness occurred and

electromyographic abnormalities characteristic of the acute phase of EAMG¹² were demonstrated.

Because of the similar patterns of binding of McAb-2 and McAb-3 to AChR from different sources (Table 1), and because these hybridomas arose from a single fusion experiment, the possibility that they might be identical molecules was examined by isoelectric focusing on polyacrylamide gels using a pH 3–10 gradient¹⁷. Antibodies were isolated from growth medium by adsorption to agarose-bound goat anti-rat Ig¹⁸, from which they were eluted in 1.0 M propionic acid. After adjustment to neutral pH and dialysis against phosphate-buffered saline, pH 7.4, and 0.02% Na₂S₂O₅, purified McAb-2 and McAb-3 antibodies bound respectively, 1.09 and 4.37×10^{-9} moles of solubilized mouse muscle AChR per mg and 3.11 and 7.24×10^{-9} moles of purified *Torpedo* AChR per mg. Unreduced, the antibodies had very similar isoelectric points (in the range of pH 7.4–7.5). However, reduction by β -mercaptoethanol revealed that the light chain bands of McAb-2 and McAb-3 (confirmed by electrophoresis in a second dimension in a SDS-polyacrylamide slab gel) were distinctly different (*pI* values 7.2 and 6.6, respectively). This indicates that McAb-2 and McAb-3 are different molecules.

Passive transfer of purified McAb-2 and McAb-3 into rats and guinea pigs induced clinical, electrophysiological and bioche-

mical evidence of EAMG (Table 3). Thus, the determinant(s) of AChR recognized by McAb-2 and McAb-3 are exposed extracellularly at the neuromuscular junction in at least three mammalian species (mouse, rat and guinea pig) and are common to all mammalian muscle AChRs examined, including man (Table 1). The fact that both antibodies bound *in vitro* to AChR complexed with ^{125}I - α -BT (molecular weight $\sim 8,000$) indicates that the antigenic determinant(s) recognized by these antibodies reside outside the receptor's binding site for ACh. Thus, using monoclonal antibodies produced by interspecies hybridomas we have established unequivocally that an autoimmune response to an antigenic determinant located outside the AChR's binding site for the neurotransmitter ACh can cause impairment of neuromuscular transmission *in vivo*. Idiotypic analysis may determine whether the same antigenic determinant of AChR is defined by these two different monoclonal antibodies. Analysis of the idiotypes of monoclonal anti-AChR autoantibodies should provide a means of defining antigenic determinants of AChR relevant to the pathogenesis and suppression of MG.

Two recent reports describe production of hybridomas secreting antibodies to AChR with specificities demonstrated only for *Torpedo* AChR^{19,20}. We believe ours to be the first report of the production of autoantibodies by hybridomas. The hybridoma technique will have important applications in the investigation of a variety of human autoimmune diseases, particularly for obtaining pathogenic antibodies of specificities which may not be detectable in serum because of quantitative absorption to target cells²¹. Hybridization of human B lymphocytes with appropriate myelomas should enable direct investigation of those specificities.

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Note added in proof: Since this article was submitted for publication it has been reported that murine myelomas hybridized with spleen cells from spontaneously autoimmune mice produced monoclonal anti-RNA²⁵ and anti-DNA²⁶ autoantibodies.

Dopaminergic activation of reticulata neurones in the substantia nigra

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Dendritic release of dopamine (DA) in substantia nigra (SN) is well established in various experimental situations¹⁻³. Morphological substrates for DA storage exist in dendrites^{4,5}, as do dendro-dendritic⁶ and dendro-axonic⁷ contacts. DA receptors in SN are located on both cells⁸ and striato-nigral terminals^{9,10}. DA is thought to regulate the activity of neighbouring dopaminergic neurones through its dendritic release by a local feedback mechanism^{11,12}. However, dendrites of DA neurones also ramify close to the neuropil of non-dopaminergic reticulata neurones in SN. The question has arisen whether dendritically released DA might also influence these neurones which, to a large extent, project to ventromedial thalamus (VM) and superior colliculus¹³. A necessary condition would be that they are sensitive to DA. In the experiments reported here this was found to be the case—a considerable proportion of nigro-thalamic neurones were activated by iontophoretically applied DA. This contrasts with its known depressant effect on pars compacta DA neurones¹² which we confirmed.

Seventeen rats (210–370 g) of both sexes were anaesthetized with chloralhydrate, 350 mg per kg intraperitoneally and additional doses given when necessary. The left VM was stimulated through a stereotactically placed bipolar electrode with 1.0–1.5 mm tip separation, using 0.1-ms pulses and currents usually of 0.1–0.5 mA (rarely up to 1.2 mA) intensity. A recording microelectrode was lowered stereotactically into the left SN. Extracellular single cell activity was monitored with glass-coated tungsten microelectrodes (exposed tips 5–10 μm long, 2.0–3.5 μm diameter), glued alongside three- or five-barrelled glass pipettes. The tungsten electrodes protruded 12–70 μm (mean 23 μm). Tip diameters of individual pipette barrels were 2.0–2.5 μm . Iontophoresis barrels contained the following substances: dopamine hydrochloride (0.2 M, pH 4.5), γ -aminobutyric acid (GABA, 0.5 M, pH 7.2), sodium glutamate (0.5 M, pH 6.7) and fluphenazine (50 mM, pH 2.4, a gift of Squibb). Sodium chloride (2 M, pH 7.0) was used for current control and balancing current. Using standard amplification methods, cell activity was recorded with a ratemeter. All cells found to be sensitive to current alone were rejected. Antidromic excitation was established when at least two of the following criteria were fulfilled: stable latency at threshold, frequency following above 500 s⁻¹, or collision with spontaneous impulses. The positions of the stimulating electrode and of each recorded cell were reconstructed histologically after small electrolytic lesions.

DA cells of pars compacta were distinguished from reticulata cells by both their histological localization and characteristic discharge pattern. They had impulses of long duration (2.71 ± 0.77 ms, mean $\pm$ s.d. compared with 1.06 ± 0.25 ms for reticulata cells; 300 Hz, -3dB filtering), in agreement with earlier reports^{13,14}. Pars compacta neurones always had a low spontaneous discharge frequency (less than 8 impulses per s) whereas reticulata neurones fired with a wide range of frequencies (2–60 impulses per s).

Of 89 reticulata cells 34 (39%) were activated by iontophoretically applied DA (at least 33% above the level of spontaneous activity), using currents of 25 to 200 nA (mean 97 ± 60 nA for 100% augmentation) (Fig. 1a–d). Inhibitory responses were not observed with reticulata neurones. Forty-eight

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reticulata neurones (55%) responded antidromically to VM thalamus stimulation with latencies of 1.75 ± 1.00 ms, in agreement with known data¹³. Of the 48 nigrothalamic neurones 16 (33%) were excited by DA. Iontophoretically applied fluphenazine, a relatively specific dopamine receptor blocker¹⁵, prevented or greatly attenuated the response to DA in seven cells, with currents of 100 or 200 nA and onset times ranging from 4 to 60 min. A clear blocking effect was not observed with four additional cells, probably because we had not recorded long enough during fluphenazine application. The responses to glutamate or GABA remained unchanged during fluphenazine application (Fig. 1d). Fluphenazine-induced DA blockade was found to be reversible in three cases.

In contrast to reticulata neurones, the spontaneous activity of pars compacta DA neurones was depressed by iontophoretically applied DA (12 of 15 neurones) (Fig. 1e). Excitation never occurred. Currents of 25 to 100 nA (mean 48 ± 22 nA) were required for a 50% reduction of activity. The DA effect was blocked by iontophoretically applied fluphenazine in all eight cases tested, in one case reversibly.

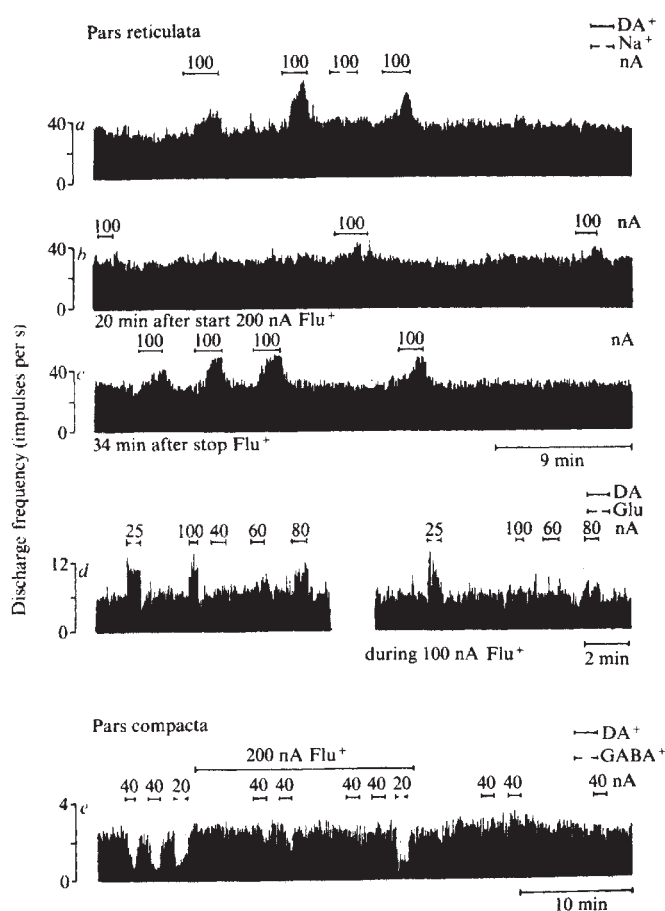


Fig. 1 Ratemeter recordings of three cells in substantia nigra. *a-c*, Recording of a cell in pars reticulata. Iontophoresis of DA⁺ increased the discharge rate (*a*). No effect of Na⁺-carried current alone. Iontophoretically applied fluphenazine (Flu⁺) greatly attenuated the response to DA (*b*); and the blockade was reversible (*c*). The onset of the drug effect was delayed because the electrode used had a large longitudinal distance (70 μ m) between the tips of the recording electrode and the iontophoresis barrels^{16,17}. (*d*) Recordings of another reticulata cell before (left) and during (right) Flu⁺ application. The response to DA was suppressed by fluphenazine, but the glutamate (Glu) effect remained unaltered, thus demonstrating the specificity of the response. Onset of right recording was 75 min after onset of Flu⁺ iontophoresis. DA and Glu were ejected with current compensation. (*e*) Activity of a pars compacta DA cell was depressed by DA. The effect was blocked during fluphenazine application but the blockade was not reversible during the time studied. Fluphenazine did not block the depressing effect of GABA.

Higher currents of DA were required to depress the activity of dopaminergic neurones than in previous studies where recording was done through one barrel of a multibarrelled pipette¹². This may have been due to the greater distance between the recording and drug applying sites in our electrodes^{16,17}. Therefore, the currents necessary for DA to activate reticulata neurones should be lower than those in our experiments when recording with multibarrelled pipettes.

The present data show a remarkable difference in the DA effects: depression of activity with pars compacta dopaminergic neurones, but activation with reticulata neurones. In striatum, DA has a predominantly depressant effect^{18,19}, but activations were also observed¹⁸. In SN, the dopaminergic neurones of pars compacta are depressed but not excited by DA¹². Neurones histologically localized in pars reticulata are so far reported to be activated as well as depressed by DA²⁰. With the additional electrophysiological criteria of this study we found that the dopaminergic and non-dopaminergic neurones of SN responded in opposite ways to iontophoretically applied DA. The mechanisms underlying these two DA effects are not yet fully understood. DA could have direct inhibitory and excitatory effects on the two types of SN cells. Alternatively, DA might activate reticulata neurones by influencing the transmitter release of afferent axonal terminals, although direct experimental evidence is as yet lacking.

Striato-nigro-thalamic connections were recently thought to function as an output of the striatum distinct from the striato-pallido-thalamic system²¹. In our study a considerable proportion of nigrothalamic neurones were found to be influenced by DA. Systemically administered amphetamine also leads to activation of reticulata neurones, and slowing of DA neurones²². This effect could well be mediated by its known DA releasing action from dendrites in SN²³⁻²⁵. Therefore, it may be envisaged that DA is released functionally from dendrites and influences reticulata neurones. In this way the DA system would have an output to VM thalamus directly at the level of SN without involving the striatum. VM projects to layer I of wide areas of rat cerebral cortex²⁶. This pattern of terminal distribution suggests a widespread, nonspecific action of impulses ascending from VM thalamus. Through its direct action on nigrothalamic cells, as suggested by our data, the DA system would exert an influence over large areas of the cerebral cortex.

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Selective development of tolerance without dependence in multiple opiate receptors of mouse vas deferens

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Studies with isolated tissues have provided strong indications for a multiplicity of opiate receptors¹⁻⁴. To satisfy the definition of cross-tolerance (tolerance to a specific opioid implying tolerance to all opioids), chronic exposure to an opiate agonist should cause tolerance and dependence in all opiate-sensitive systems. On the other hand, if multiplicity of opiate receptors is defined in terms of multiple recognition sites whose activation by specific opioids cause independent responses, then development of cross-tolerance is less likely. The mouse vas deferens (MVD) provides a useful model to test these considerations. This tissue contains δ -opiate receptors, for which D-Ala²-D-Leu⁵-enkephalin (DADL) represents the most selective agonist, as well as μ -receptors, for which sufentanyl appears highly selective^{3,5-7}. We report here that isolated vasa deferentia of mice chronically treated with DADL are 800-fold less sensitive to this δ -receptor agonist, whereas there is no cross-tolerance to μ -receptor agonists. On the other hand, chronic exposure of mice to sufentanyl renders their vasa deferentia more than 1,000-fold less sensitive to sufentanyl, but affects their sensitivity to DADL insignificantly. Apparently, such highly tolerant preparations exhibit no detectable sign of dependence when challenged with naloxone or clonidine. These findings favour the notion that opiate tolerance and dependence dissociate in the MVD.

Mice (NMRI, 25 g) were treated chronically from osmotic minipumps with either DADL or sufentanyl. Vasa deferentia were removed from the animals and set up for electrical stimulation⁴. To prevent unmasking of opiate receptors, the bathing solution contained DADL or sufentanyl at the concentration measured in the serum. After equilibration dose-response curves were established and the drug concentrations required to inhibit electrically evoked twitches by 50% were determined (IC_{50}). Tests for dependence were conducted in the presence of the narcotic agonist using naloxone as antagonist.

The effect of chronic DADL infusion on vasa deferentia is shown in Fig. 1. Dose-response curves for DADL were shifted to the right, indicating development of tolerance (Fig. 1a). The degree of the shift was dependent on the amount of DADL infused—administration of 5 μ g DADL per h for 6 days resulted in an 800-fold shift (IC_{50} 4×10^{-7} M; naive 5×10^{-10} M). Vasa deferentia taken from DADL-treated animals, which were set up in the presence of 20 nM DADL, displayed a similar twitch tension evoked by electrical stimulation as compared to naive tissues in the absence of any drug. Washing highly tolerant preparations (5 μ g DADL per h, 6 d) for 3 h with drug-free Krebs-Ringer solution increased the sensitivity to DADL slightly (IC_{50} 1×10^{-7} M) and the sensitivity was not further increased by a 5-h wash procedure. In vasa deferentia taken from mice infused with 5 μ g DADL per h for 24 h, only a 200-fold shift of the dose-response curve was found (IC_{50} 8×10^{-8} M). Washing these preparations for 2 h with drug-free Krebs-Ringer solution resulted in a considerable loss of tolerance (IC_{50} 9×10^{-10} M). Figure 1b shows dose-response curves for sufentanyl, normorphine and clonidine on vasa deferentia of naive and DADL-infused (5 μ g per h, 6 d) mice. Although the vasa deferentia are highly tolerant to the δ -receptor agonist DADL, the IC_{50} for normorphine was not

changed and only a fourfold increase was observed for sufentanyl. As opiate dependence is associated with subsensitivity to clonidine, additional tests were conducted with this α -adrenoreceptor agonist⁸. The IC_{50} value of clonidine was not changed in tolerant preparations challenged in the presence of naloxone (200 nM).

Figure 2 shows dose-response curves for sufentanyl and DADL on vasa deferentia from untreated mice and from mice infused for 6 d with sufentanyl at a delivery rate of 10 μ g per h. Again, to prevent unmasking of opiate receptors *in vitro*, the tissues of chronically treated mice were set up in the presence of 5 nM sufentanyl, the corresponding serum concentration. Sufentanyl was over 1,000-fold less potent (IC_{50} 50 nM) in vasa deferentia taken from sufentanyl-treated animals as compared to naive preparations (IC_{50} 0.04 nM). A high degree of cross-tolerance with normorphine was found in such preparations. In contrast, DADL showed identical activities in control and sufentanyl-tolerant vasa deferentia, as does, for example, Leu-enkephalin (details to be published elsewhere).

Highly tolerant vasa deferentia from mice chronically infused with DADL (5 μ g per h, 6 d) were challenged with naloxone (50–1,000 nM) in the presence of 20 nM DADL. Neither basic tension (absence of electrical stimulation) nor electrically evoked twitch tension was affected by the narcotic antagonist. However, naloxone completely antagonized the inhibition of

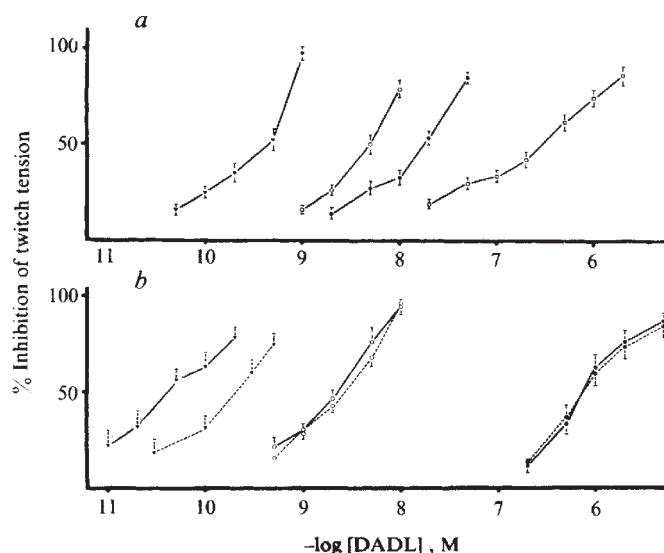


Fig. 1 a, Dose-response curves for D-Ala²-D-Leu⁵-enkephalin (DADL) on the electrically stimulated isolated mouse vas deferens (0.1 Hz, 0.5 ms, 40 V; basic tension 0.05 g). Data are for preparations from untreated mice (▼) and from mice infused with 0.5 μ g DADL per h (○), 1.5 μ g per h (●) and 5 μ g per h (□) for 6 d. Infusion was from osmotic minipumps (Alza model 1701) implanted subcutaneously, which release 1 μ l per h. The preparations taken from chronically DADL-treated mice were incubated *in vitro* with their corresponding serum DADL concentrations, which was 1 nM in mice receiving 0.5 μ g per h, 3 nM in mice infused with 1.5 μ g per h and 20 nM during infusion of 5 μ g per h as determined in the isolated vas deferens. These data are the means of three to five serum samples from different mice assayed the 2nd, 4th and 6th days of the experiment. The maximal standard error of the mean serum data was <15%. b, Dose-response curves for sufentanyl (▽), clonidine (○) and normorphine (●) on vasa deferentia taken from non-pretreated mice and from mice infused for 6 d with 5 μ g DADL. Preparations from chronically treated mice were set up for electrical stimulation in the presence of DADL (20 nM). Dotted lines represent curves obtained from vasa deferentia of infused mice. A period of 30 min was allowed for equilibration after set-up in the presence of the enkephalin derivative with changes of the bath fluid every 5–10 min. Each point on the figure is the mean of at least six tests on tissues from different animals. Vertical bars indicate standard errors.

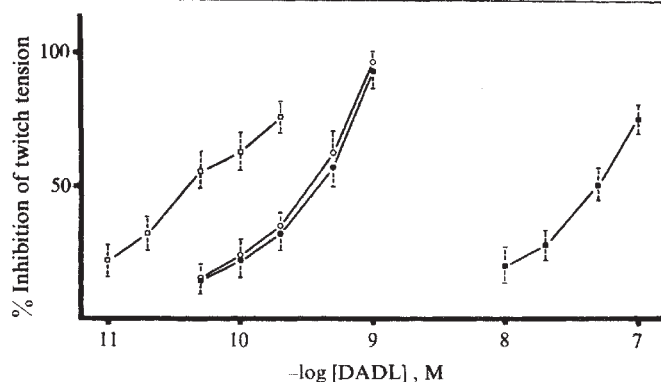


Fig. 2 Dose-response curves for sufentanil (squares) and DADL (circles) on vasa deferentia of naive mice (open symbols), and from those infused for 6 days with 10 µg sufentanil per h (solid symbols). The preparations of chronically treated mice were set up in the presence of 5 nM sufentanil, the respective drug concentrations in the serum at the time of killing. Each point is the mean of at least eight determinations on tissues of different animals. Vertical bars represent s.e.m.

twitch tension produced by 10^{-6} M DADL, without an overshoot increase in tension. Similarly, no signs of withdrawal were demonstrated in sufentanil-tolerant preparations.

The findings reported here unequivocally point to a multiplicity of opiate receptors. Selective activation of a specific population of opiate receptors, δ -receptors, for prolonged periods causes their selective desensitization (that is, tolerance), leaving the responsiveness of μ -receptors unaffected. By analogy, tolerance of the μ -receptor develops upon their chronic activation without affecting the responsiveness of δ -receptors. Thus, these data conflict with the classical notion that cross-tolerance reflects a general characteristic of opiates. The lack of cross-tolerance between δ -receptor agonists and μ -receptor agonists does not exclude, however, a mutual receptor activation at sufficiently high concentrations of these compounds.

One aspect of the present investigations relates to the phenomenon of dependence, which has been postulated to be invariably accompanied with tolerance^{9,10}. Although the vasa deferentia exhibited an extremely high degree of tolerance, no signs of dependence were observed on challenge with naloxone or clonidine. The response to each of the drugs is considered a reliable test for dependence in opiate-tolerant tissues from the myenteric plexus of the guinea pig^{8,11}. Although a dissociation of tolerance and dependence conflicts with the common understanding of the mechanisms underlying these phenomena¹²⁻¹⁴, an adaptation to chronic opiate receptor activation by a reduction of opiate binding sites¹⁵ could explain our findings. Moreover, we have some evidence that chronic DADL infusion in mice results in a reduction of the number of δ -receptors in the MVD. Such a mechanism of adaptation resembles that involved in desensitization of neurohormone receptors on chronic activation¹⁶⁻¹⁸.

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Dopaminergic stimulation of prolactin release

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Prolactin (PRL) secretion from anterior pituitary is believed to be under tonic inhibitory control of dopamine (DA)¹ released from the tubero-infundibular dopaminergic neurones into the hypophysial portal blood^{2,4}. Inhibition of PRL release by DA seems to be mediated by stereospecific DA receptors located in PRL cells^{5,6}. Apomorphine and various ergot alkaloids such as bromocriptine mimic the inhibitory effect of DA both *in vivo* and *in vitro*, presumably by a direct agonist action on these 'inhibitory' receptors^{1,6,7}. We now report that PRL secretion in primary cultures of rat pituitary cells can be stimulated by DA when concentrations a thousand times lower than those required for inhibition are used. Secretion rates above basal release can also be induced by apomorphine and bromocriptine when the 'inhibitory' receptors are blocked with certain DA receptor antagonists.

Rat pituitary cells from adult male Wistar rats (300-350 g) maintained in monolayer culture^{7,8} and superfused pituitary cells cultured on polystyrene beads (Biosilon, Nunc) were used. All tests were done after a period of 5 days in culture. PRL was measured using a specific rat PRL radioimmunoassay using the kits and procedures from the NIAMDD Pituitary Hormone Distribution Program, Bethesda, Maryland. Statistical analysis of the data was by multiple *t*-test.

Figure 1 shows the effect of DA in both these cell systems. In monolayer cultures picomolar concentrations of DA stimulated PRL release by more than 50% ($P < 0.001$), whereas inhibition occurred from concentrations about a thousand times higher. In superfused cells there was an 82% rise in average secretion rates during superfusion with 1-100 pM DA, compared with maintained average secretion rate during superfusion with the control medium ($P < 0.001$). Average secretion rates were 3.78 ± 0.15 ng min⁻¹ and 2.08 ± 0.12 ng min⁻¹, respectively (mean $\pm$ s.e.m.). When DA was superfused together with 10 nM of the DA receptor antagonists haloperidol or (+)butaclamol, stimulation was 56% and 107% above basal release, respectively. Thus, stimulation by DA seemed to be partially antagonized by a 'low' concentration of haloperidol but not by (+)butaclamol.

Apomorphine was more potent than DA in suppressing PRL release (EC₅₀ 0.95 nM and 14 nM, respectively) but, as shown in Fig. 2, it had only a marginal, if any, stimulatory effect. However, when co-incubated with 10 nM of the DA receptor antagonist haloperidol, picomolar concentrations of apomorphine stimulated PRL release to ~30% above basal secretion rates ($P < 0.01$); such stimulation was not seen when haloperidol was added alone. Similar results were obtained in superfused cells. Picomolar concentrations of apomorphine also stimulated PRL release when co-incubated with 10 nM domperidone or

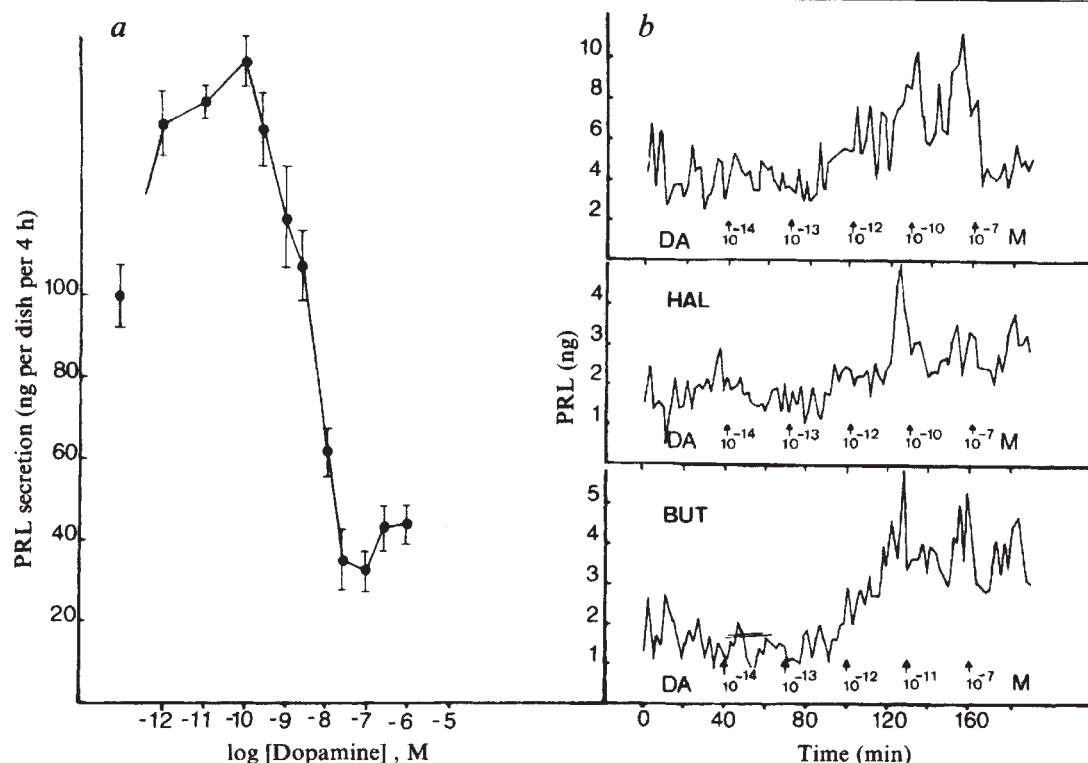


Fig. 1 Effect of dopamine (DA) alone or together with 10 nM haloperidol (HAL) or (+)butaclamol (BUT) on PRL secretion from primary cultures of dispersed rat pituitary cells (male Wistar rats, 300–350 g). The amount of PRL secreted in the medium is expressed in ng equivalents of the reference preparation NIAMDD rat-PRL-RP-1. *a*, Monolayer cultures. The culture method was as described previously^{7,8}. Cells were plated in multiwell Petri plates (Falcon 308) at 2×10^5 cells per well. Culture medium was Dulbecco's modified Eagle's medium (DMEM), supplemented as before⁸ with 10% horse serum, 2.5% fetal calf serum, non-essential amino acids (all from Gibco) and antibiotics. Tests were performed on the fifth day in culture. The cultures were washed with Earle's balanced salt solution (EBSS) (Gibco), preincubated in EBSS for 1 h at 37 °C in a CO₂/air incubator, washed again and incubated for 4 h at 37 °C in a CO₂/air incubator in 500 μ l EBSS,

pH 7.6, containing the test substances. The various concentrations of DA (Sigma), freshly dissolved and further diluted in 1% ascorbic acid and of the DA receptor antagonists (Janssen) dissolved and further diluted in ethanol were added in 5- μ l aliquots. Controls received the vehicles alone. After incubation the media were collected, bovine serum albumin (BSA) added to a final concentration of 0.1%, centrifuged and the supernatants stored at -25 °C until assayed. Each value represents the mean $\pm$ s.e.m. of 6 replicate wells. *b*, Superfused cell cultures. Dispersed pituitary cells suspended in 1 ml culture medium were allowed to settle on polystyrene beads (Biosilon, Nunc average diameter 330 μ m) packed in a 1-ml sterile syringe the bottom of which was closed with a 25- μ m nylon mesh. Homogenous distribution was obtained by pumping the cell suspension through the column of beads at low flow rate (1 ml h⁻¹). The beads had been pretreated with poly-L-lysine^{29,30} to ensure instant and irreversible attachment of the cells^{8,29}. Approximately 10^5 – 1.5×10^6 cells were loaded on 2×10^5 beads. After washing with the same suspending medium the beads were gently transferred to an untreated plastic Petri dish and maintained in culture medium (see *a*). After 5 days in culture microscopic examination showed that the cells had made individual monolayers over the bead surface, although small aggregates of cells were seen on some beads. The superfusion system consisted of a small plastic column (diameter 5 mm), the bottom of which was closed with a 25- μ m nylon mesh and connected to a peristaltic pump. The column and connecting tubings were mounted in a waterbath at 37 °C. To minimize adsorption of drugs, plastic containers, columns and connecting tubings were rinsed before each experiment with 1% BSA in phosphate-buffered saline. A number of beads, containing ~ 3 – 4×10^6 cells, were packed on the bottom of each column and the peristaltic pump superfused the beads with DMEM at a flow rate of 1 ml min⁻¹. The volume of medium covering the beads was maintained at 1 ml. Before the control and test substances were added (dissolved and diluted as in *a*), the cells were allowed to equilibrate for 40–60 min with DMEM. Subsequently, 2-ml fractions were collected every 2 min, BSA added as in *a* and the samples stored at -25 °C until assayed.

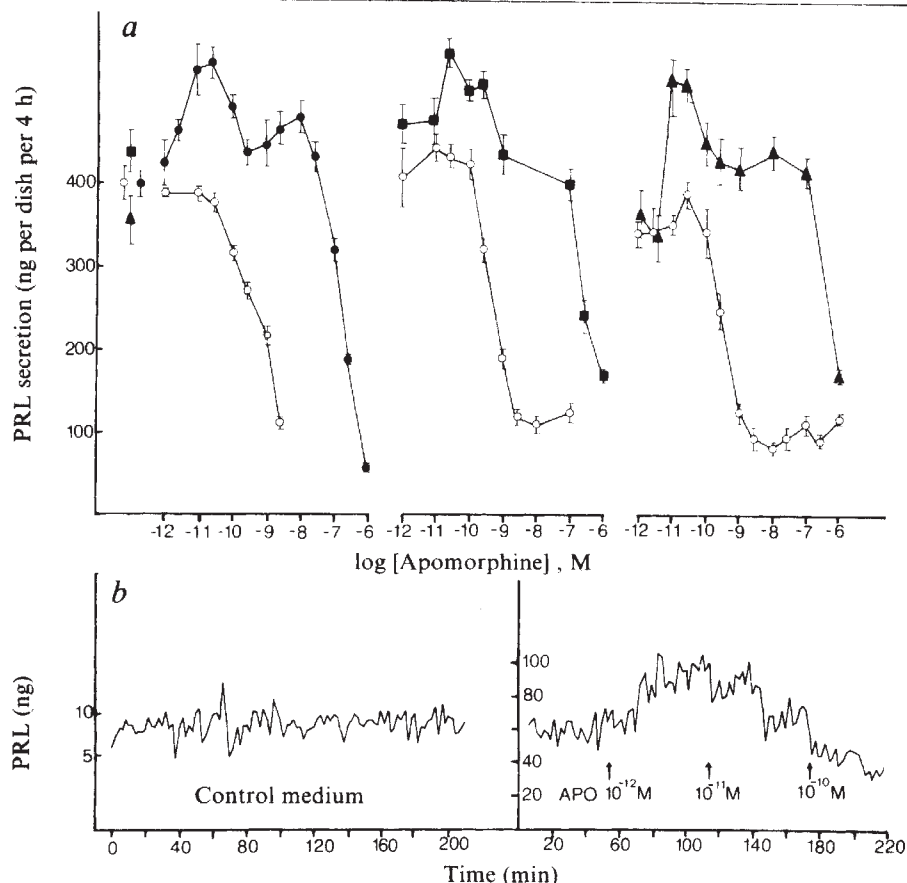
100 nM haloperidol, a substituted benzamide structurally related to the butyrophenones but with a distinct pharmacological profile⁵. As expected^{1,6,7}, the DA receptor antagonists completely prevented inhibition by nanomolar concentrations of apomorphine. Thus, it is reasonable to assume that the DA receptor antagonists potentiate the stimulatory action of apomorphine because at the concentrations used they completely block the 'inhibitory' DA receptors but do not, or only partly, block those receptors which mediate stimulation of PRL release. Note that maximal stimulation by apomorphine was seen at concentrations similar to those of DA but that, in contrast to DA, the magnitude of the effect was less, and slightly higher concentrations even antagonized the stimulatory effect. This seems to indicate that apomorphine has a lower intrinsic activity than DA. Moreover, stimulatory concentrations of apomorphine (10–100 pM) were closer to inhibitory concentrations (0.25–10 nM) than was the case with DA (1–100 pM against 1–100 nM) and this may explain why apomorphine, when applied alone, was not capable of enhancing secretion.

As shown in Fig. 3, bromocryptine was extremely potent in suppressing PRL release. Concentrations as low as 10^{-18} M were already effective. Co-incubation with 0.1 or 1 nM haloperidol or domperidone shifted inhibition by bromocryptine towards higher concentrations but, from about 10^{-12} M bromocryptine, PRL release again increased ($P < 0.01$ or 0.001). When incubated with 10 nM of the DA antagonists, bromocryptine stimulated PRL release above basal secretion rates

($P < 0.001$). This occurred at concentrations entirely overlapping with the inhibitory concentrations.

These experiments show that, in addition to their inhibitory effects on PRL release, DA and the DA receptor agonists apomorphine and bromocryptine have a stimulatory potency. In 5-day-old rat pituitary cell cultures, DA stimulated PRL release at concentrations as low as 1 pM, about 1,000 times lower than concentrations that cause inhibition. Picomolar concentrations of apomorphine and even lower concentrations of bromocryptine were also stimulatory, although this effect was seen only when inhibition was prevented by certain DA receptor antagonists. As apomorphine and bromocryptine are highly specific ligands of DA receptors^{10,11}, these findings strongly suggest that the 'stimulatory' receptors are specific DA receptors. Moreover, data indicate that these DA receptors are different from receptors that mediate inhibition. First, the relative stimulatory potencies of DA, apomorphine and bromocryptine were different from those for inhibition. DA stimulated PRL release from concentrations considerably lower than those causing inhibition, whereas apomorphine and bromocryptine stimulated release at concentrations closer to or overlapping inhibitory concentrations. Second, stimulation by DA could be antagonized, at least partially, by 10 nM haloperidol, but not by 10 nM (+)butaclamol, a DA receptor antagonist that is more potent than haloperidol in preventing the dopaminergic inhibition of PRL release^{6,7}. It might be argued that stimulation is mediated by the inhibitory receptor in a different functional

Fig. 2 Effect of apomorphine alone (○) or together with 10 nM haloperidol (●), domperidone (■) or haloperidol (100 nM) (▲) on PRL secretion from primary cultures of dispersed rat pituitary cells. Methods as in Fig. 1. *a*, Monolayer cultures, means $\pm$ s.e.m. of six replicate wells. *b*, Superfused cells.



state which would exist only at low receptor occupancy. However, if this were so, it would be difficult to explain apomorphine stimulation in conditions where virtually all inhibitory receptors were occupied by a DA receptor antagonist. Thus, the action of DA on PRL release rather involves two different receptors or receptor sites.

Evidence for more than one DA receptor type has already been given for a number of tissues, particularly the brain^{10,12-14}

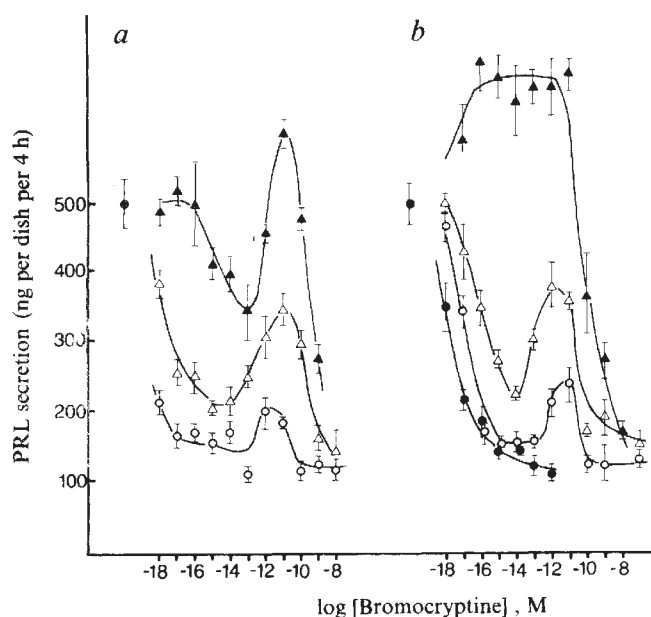


Fig. 3 Effect of bromocryptine alone (●) or together with: *a*, 0.1 nM (○), 1 nM (Δ) and 10 nM (▲) haloperidol; or *b*, domperidone, on PRL release from primary cultures of dispersed rat pituitary cells. Monolayer culture method as in Fig. 1. Bromocryptine (Sandoz) was dissolved and further diluted in ethanol. Means $\pm$ s.e.m. of six replicate wells.

Various receptor categories have been recognized on the basis of: their differential specificity for DA receptor agonists and antagonists^{10,15-20}; differential sensitivity of behavioural^{11,21,22} and electrophysiological²³ responses to DA agonists; adenylyl cyclase activation^{10,24}; and regulation by guanine nucleotides²⁵. Recent work by Sibley and Creese has suggested that there may be a DA receptor in the anterior pituitary distinct from the receptor that mediates inhibition of PRL release²⁶. This DA receptor seems to be regulated by guanine nucleotides and is possibly linked to adenylyl cyclase. It is tempting to hypothesize that this receptor is involved in stimulation of PRL release by DA. However, the fact that DA agonist activity at this receptor is exerted at micromolar concentrations²⁶ is difficult to reconcile with the finding here that stimulation of PRL release is evoked at picomolar concentrations. By contrast, the receptors involved in stimulation of PRL release seem to have certain characteristics in common with the DA 'autoreceptors' on cell bodies or terminals of nigrostriatal dopaminergic neurones²³: the latter receptors are much more sensitive to DA²³ than adenylyl cyclase-linked postsynaptic DA receptors in the striatum²³; apomorphine and bromocryptine both appear to be agonists of these receptors^{11,19,22,23}; and haloperidol is a considerably more potent antagonist than (+)butaclamol¹⁹. Thus, certain characteristics of DA autoreceptors seem to correspond with those of pituitary receptors involved in dopaminergic stimulation of PRL release.

Thus the data presented here suggest that dopaminergic control of PRL secretion at the level of the PRL cells may involve two different receptors for DA, one being inhibitory and the other stimulatory. This is most intriguing in view of the fact that PRL release is believed to be under tonic inhibitory control by DA¹. The average concentration of DA reported in hypophyseal portal blood of male rats is 0.25 ng ml⁻¹ (about 1.3 nM)⁴. This concentration is far below that required for maximal inhibition of PRL release *in vitro* as well as *in vivo*²⁷ and close to the stimulatory concentrations found here. At such a concentration it is likely that the effect of DA on PRL secretion

depends on the relative contribution of stimulatory and inhibitory effects. It seems, therefore, that the role of DA in control of PRL secretion is not just one of tonic inhibition. Changes in the relationship between 'stimulatory' and 'inhibitory' receptors could be one of the mechanisms underlying the dynamic regulation of PRL release by DA.

Part of this work has been presented elsewhere²⁸. We thank Dr P. A. J. Janssen and Dr J. E. Leysen for discussions and gifts of dopamine antagonists, Dr A. F. Parlow and the Pituitary Hormone Distribution Program of NIAMDD, Bethesda, Maryland for rat-PRL radioimmunoassay kits, and Miss M. Bareau for typographical work. This work was supported in part by an I.W.O.N.L. grant.

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Inhibin is absent from azoospermic semen of infertile men

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There is ample evidence that the secretion of follicle stimulating hormone (FSH) by the pituitary gland is regulated by a non-steroidal hormone of gonadal origin, termed inhibin^{1–3}. In several species, testicular extracts⁴, gonadal lymph⁵ and seminal plasma⁶ have been shown to contain proteins which inhibit FSH release; lack of suitable assays for inhibin has, however, prevented clear definition of its physiological significance. Men whose testes show the histological changes of germinal cell failure usually have raised FSH levels in the blood, but may have normal levels of luteinizing hormone (LH) and testosterone⁷. Such patients would thus be predicted to show reduced inhibin production. To test this, we have measured the FSH inhibitory activity of seminal plasma from azoospermic subjects with raised plasma FSH levels. We report here our confirmation of diminished inhibin levels in seminal plasma of these patients, thus providing convincing support for its physiological role as a modulator of FSH production in man.

Our subjects were eight patients with idiopathic azoospermia and raised serum FSH levels, who were attending the Infertility Clinic at Prince Henry's Hospital (Table 1). Testicular histology of three of these patients showed changes of Sertoli-cell-only syndrome, seminiferous tubule hyalinization and moderate hypospermatogenesis, respectively. Levels of LH, although higher than in eight control men with normal semen analyses, were within the laboratory normal range (0.6–3.6 IU l⁻¹). For the purpose of this study, we considered a normal semen analysis to comprise a sperm count >20 × 10⁶ per ml, sperm motility >60% and normal sperm morphology. FSH inhibitory activity was also measured in semen from three patients with non-functioning testicular tissue. One patient with Klinefelter's syndrome had testicles of approximately 1 cm³ (plasma FSH 30.2, LH 9.4 IU l⁻¹). The other two patients were found at operation to have small bilateral abdominal testicles. Plasma FSH levels were 17.0, 23.7 IU l⁻¹ and LH levels 9.4, 4.2 IU l⁻¹. Neither was on androgen replacement treatment at the time of collection of semen samples preoperatively. Seminal plasma collected from six patients between 2 and 8 months post-vasectomy was used as an azoospermic control as vasal

obstruction does not affect the integrity of the seminiferous epithelium.

Semen samples were centrifuged twice at 3,000g for 30 min and the plasma separated. An equal volume of charcoal (Merck), 200 mg ml⁻¹ in phosphate-buffered saline (CSL, Melbourne), was added to each sample. After allowing the samples to stand for 24 h at 4 °C, the charcoal was removed by two centrifugations at 4,000g for 30 min at 4 °C. Charcoal was used to remove poorly defined small molecular weight substances that seem to interfere with the assay by causing nonspecific toxic changes.

Inhibin-like activity was measured in combined, multiple parallel line bioassays^{8,9}, in which the unknowns were expressed in terms of an inhibin standard derived from ovine testicular lymph (OTLP)¹⁰, arbitrary potency 1 U per mg. The inhibin standard was added to dispersed rat anterior pituitary cell cultures over a dose range of 0.156–2.5 mg ml⁻¹ (Fig. 1). Semen samples were assayed in quadruplicate at four or five dilutions up to a maximum concentration of 50 µl seminal plasma per ml of incubation medium. At the end of the 72-h incubation period, the cells were lysed and FSH and LH were measured in the lysate (Fig. 1 legend)⁹. Lack of change of LH content was used as an index of the specificity of the inhibitory response (Fig. 1).

Table 1 Sperm counts, FSH, LH and testosterone levels* in eight normal and eight infertile patients

Group	n	FSH (IU l ⁻¹)	LH (IU l ⁻¹)	Testosterone (nmol l ⁻¹)	Sperm count (10 ⁶ ml ⁻¹)
Azoospermic	8	11.7 (6.8–17.0)	2.3 (1.5–3.8)	20.0 (15.0–24.0)	0
Normal	8	2.1 (1.5–2.9)	1.0 (0.7–1.8)	22.0 (13.0–42.0)	73 (22–170)

* Median (and range).

In the eight normal subjects, dose-response lines were significantly parallel to the reference standard; the samples had relative potencies of 20.8–60.0 U ml⁻¹ of inhibin activity (Fig. 2). In men with azoospermia (Fig. 2), the inhibin activity in the seminal plasma was undetectable (<2.5 U ml⁻¹ in five men) or low (<6.3 U ml⁻¹ in three men). A dose-response curve could not be obtained, because addition of seminal plasma at concentrations above 50 µl ml⁻¹ to pituitary cell cultures was often followed by morphological changes, suggesting a toxic effect, and a concomitant reduction of LH content. No FSH inhibitory activity was detectable in the semen of the three patients with testicles of small size. The six post-vasectomy samples had

measurable inhibin-like activities ranging from 4.5 to 20.2 U ml⁻¹ (mean 15.5 U ml⁻¹, s.d. 5.8 U ml⁻¹), significantly less than for normal subjects although higher than found in patients with germ cell failure.

To exclude the possibility that azoospermic seminal plasma in these patients contained a factor blocking the action of inhibin, we added inhibin derived from OTLP or human follicular fluid to azoospermic seminal plasma. In both cases, the predicted inhibition of cell content of FSH was observed, without significant change of the dose-response curve.

Although a considerable body of data suggests the existence of a non-steroidal regulator of pituitary FSH secretion¹⁻³, the relationship exists between the cell population of the epithelium spontaneous disorders of the seminiferous epithelium has not been studied. It has been previously shown that a reciprocal relationship exists between the cell population of the epithelium and serum FSH, and that serum FSH levels are uniformly elevated only in the presence of severe seminiferous tubule damage¹¹. In the absence of a well characterized purified preparation of inhibin, physiological studies must rely on specific

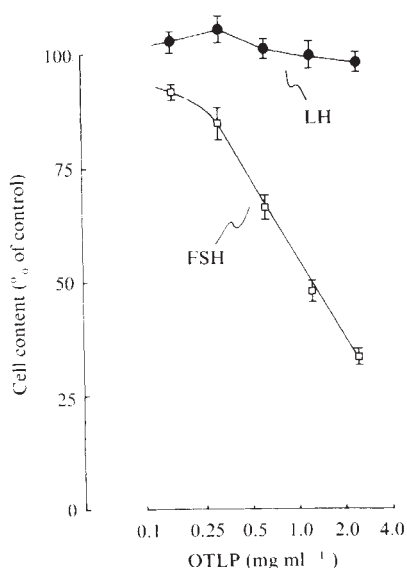


Fig. 1 FSH and LH content in 22 experiments (mean $\pm$ s.e.m.) using OTLP standard as the inhibin source. Similar dose responses of FSH, with lack of LH change, were observed after addition of treated normal human seminal plasma. Dispersed pituitary cells were prepared by enzymatic methods in sterile conditions^{9,10}. Aliquots (0.5 ml) of medium containing 125,000 cells were added to each well of tissue culture plates (Coster, 3524) and incubated for 24 h at 37 °C in 5% CO₂, 95% air before adding semen samples and OTLP standard. All preparations were added in quadruplicate at four or five dilutions. After a further 72 h in culture, the medium was aspirated and discarded, and 0.5 ml of phosphate buffer containing 0.1% Triton X-100 was added to each well. This causes detachment and lysis of cells, releasing intracellular hormone into the buffer. Standards for hormones measured in the lysate were rat FSH-RP1 and rat LH-14 (NIAMDD)^{9,12}. The FSH and LH concentrations were expressed as % of control (no added inhibin) and regression analysis of the FSH dose-response lines carried out by computer, calculating slope (b), index of precision (λ) and significance of regression (Finney's g)¹³. In 22 experiments using the reference standard (OTLP): $-b = 24.0$ (19.2–31.4), $-\lambda = 0.06$ (0.032–0.110) and Finney's $g = 0.01$ (0.003–0.057). Median and range are given. Interassay variation of three unknown preparations was 20.1%, 19.8% and 15.0%. Specificity is satisfactory as changes of rat LH, GH or prolactin were not significant⁹. Bioassay potency estimates of unknown preparations were similar using the more conventional methods of *in vitro* assay measuring LH-releasing hormone stimulated release⁹. From eight separate experiments, four wells per experiment, the FSH content (of control wells) at the end of 96 h culture was 636 ± 28.0 ng per 125,000 cells.

bioassay. The present results have been obtained with a reproducible assay in which the cell content of FSH, but not LH, is decreased by inhibin-containing preparations⁹. In men with severe seminiferous tubule disease, as shown by elevated levels of serum FSH and azoospermia, seminal plasma levels of inhibin are low or undetectable. These patients had normal peripheral plasma testosterone concentrations, suggesting that the elevations of serum FSH represent a release from the negative feedback influence of this inhibitor.

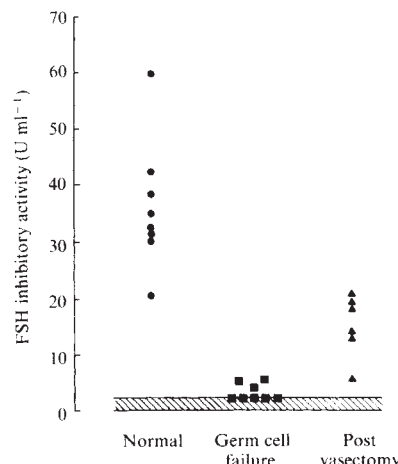


Fig. 2 FSH inhibitory activity in human seminal plasma of normal, post-vasectomized and azoospermic infertile patients. The assay limit for measurement of inhibin was 2.5 U ml⁻¹ (indicated by shading).

Seminal plasma of vasectomized patients contained FSH inhibitory activity; this suggested secretion and perhaps concentration of inhibin in the prostatic and seminal vesicular fluid. However, this does not imply extragonadal production of this inhibitor, because the levels of inhibin were low in infertile subjects despite the presence of normal concentrations of testosterone, the major factor maintaining prostatic function. Therefore, the likely source of this material is the testis, as the only difference between groups was the integrity of the germinal epithelium. Further support for the testicular production of this inhibitor is provided by the observation that no inhibin was present in the semen of the two subjects with abdominal testes or the patient with Klinefelter's syndrome. Such findings suggest a causal relationship between seminiferous tubular disease and decreased production of inhibin.

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Note added in proof: A substance which diminished FSH levels in castrated male rats was found to be present in the seminal plasma of normal and oligospermic subjects, but was absent in subjects with azoospermia caused by inhibition of gametogenesis¹⁴.

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Renin-like effects of NGF evaluated using renin-angiotensin antagonists

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Intracranial injection of angiotensin II (AII) or activation of the cerebral isorenin-angiotensin system with intracranial renin causes an immediate thirst¹ and a delayed sodium appetite² in the rat. Nerve growth factor (NGF), a polypeptide trophic factor for peripheral sympathetic and sensory neurones³, has also been reported to be a potent stimulus to thirst⁴ and sodium appetite⁵ when injected into the brain of the rat. Lewis *et al.*⁵ drew attention to the marked similarity between the effects of 2.5S NGF and renin on thirst and sodium appetite and suggested that the NGF responses were mediated by the cerebral isorenin-angiotensin system. We report here that NGF-induced thirst and sodium appetite, as well as increased blood pressure and increased ornithine decarboxylase activity in the brain and liver, depend on the formation of AII (see also ref. 6).

To test the hypothesis that NGF-induced thirst and sodium appetite depend on the activation of the cerebral isorenin-angiotensin system⁵, rats were pretreated with saralasin, a competitive antagonist of AII (ref. 7), or the converting enzyme inhibitors^{8,9}, SQ 20881 or SQ 14225. These inhibitors are effective in suppressing the thirst¹⁰ and sodium appetite (unpublished results) elicited by intracranial administration of renin.

Intracranial injection of saralasin caused a dose-dependent inhibition of water intake induced by injection of 0.66 µg 2.5S NGF¹¹ through the same cannula (see Fig. 1 legend for method). In 1 h the intake of water after NGF injection was 15.3 ± 1.5 ml (*n* = 18), and this was reduced by saralasin pretreatment (1, 5 and 25 µg respectively) to 9.2 ± 2.6 ml (*n* = 8, *P* < 0.05), 6.3 ± 0.4 ml (*n* = 4, *P* < 0.01), and 4.4 ± 1.5 ml (*n* = 6, *P* < 0.001). The 1-h intake of 2.7% NaCl after NGF injection (2.4 ± 0.7 ml, *n* = 18) was completely abolished by 25 µg saralasin (*n* = 6, *P* < 0.05).

Intracranial injection of SQ 20881 (25 µg) or SQ 14225 (5 µg) resulted in complete inhibition of water intake during the first hour after administration of 0.66 µg 2.5S NGF through the same intracranial cannula (Fig. 1). The 18-h water and 2.7% NaCl intakes after NGF were unaffected by the single injection of 25 µg SQ 20881, but were attenuated significantly by the single injection of 5 µg of the more potent converting enzyme inhibitor, SQ 14225.

The effect of converting enzyme inhibition on the overnight intakes of water and 2.7% NaCl after NGF was further studied by infusing SQ 20881 throughout the 18 h of the experiment. Continuous infusion of the inhibitor resulted in a significant depression of the overnight water and 2.7% NaCl intakes after NGF injection (Fig. 1). The effect of NGF on thirst and sodium appetite is therefore indistinguishable from that of renin with respect to its dependence on generation of AII.

The renin-like activity of NGF preparations was further tested by measuring their pressor activity. Intravenous administration of dipotentially active 7S NGF¹² produced a dose-dependent rise in arterial blood pressure (Fig. 2), as did 2.5S NGF (data not shown). The pressor responses to NGF were similar to those of equipotent doses of renin. The responses showed a slow onset over 1–2 min and lasted for up to 20 min. They were enhanced by nephrectomy and were completely abolished by pretreatment with 25 µg saralasin, 25 µg SQ 14225, or NGF antiserum¹³. In contrast, immunogenically

pure β NGF subunit¹⁴ was devoid of pressor activity, and also had a smaller and more variable effect on thirst and sodium appetite compared with other preparations of NGF.

These findings raise the possibility that other biological effects of NGF are caused by AII formed as a result of the action of the renin-like enzyme in NGF preparations. NGF has recently been reported to produce an increase in the activity of ornithine decarboxylase (ODC) in a variety of tissues^{15,16}, including brain¹⁷ and liver¹⁸. In the present experiments intraventricular administration of either 7S NGF or renin was followed 6 h later by an increase in ODC activity in the brain and liver (Fig. 3). The enzyme induction and water intakes following intraventricular administration of either NGF or renin were reduced significantly by pretreatment with 35 µg SQ 14225. The dependence of the enzyme induction in brain and liver on AII formation is consistent with the recent finding that large doses of AII are an effective stimulus to ODC induction in these tissues¹⁹. Induction of ODC in the liver following NGF administration depends on activation of the pituitary-adrenal axis¹⁸. Furthermore, administration of NGF²⁰, like AII (refs 21–23), causes an increase in plasma ACTH and corticosteroids. Therefore, stimulation of ACTH by NGF preparations may depend on their content of renin-like enzymes.

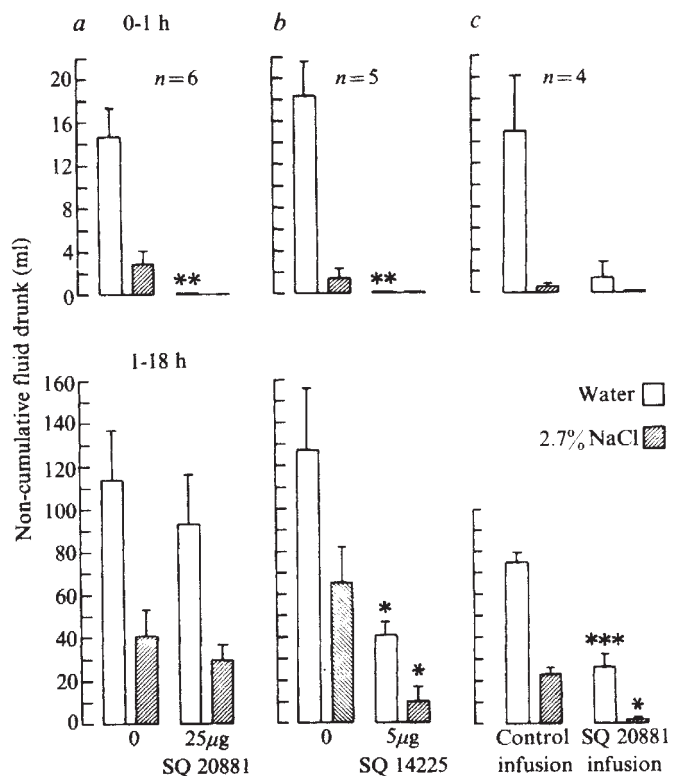


Fig. 1 The effect of angiotensin converting enzyme inhibitors on NGF-induced thirst and sodium appetite. Five min before injection of 2.5S NGF into the preoptic area or third ventricle, rats were given intracranial injections (1 µl) of 0.9% NaCl (0, vehicle), or SQ 20881 (Bachem) (a), or SQ 14225 from Dr Z. P. Horowitz, Squibb (b). For intracranial infusions into the third ventricle (c), rats were first infused with either 3 µl of 0.9% NaCl (control) or 3 µl of 0.9% NaCl containing 25 µg SQ 20881 at the rate of 1 µl every 3 min. The injector was then removed and 0.66 µg 2.5S NGF in 4 µl 0.9% NaCl was aspirated into the tubing. The injector was replaced and the rats were infused with the solution at the same rate. After 12 min the infusion rate was changed so that the rats received 25 µg SQ 20881 every hour for 18 h. Control rats received 3 µl per h of 0.9% NaCl during this period. Water and 2.7% NaCl intakes were measured at 1 and 18 h after the start of the infusion of NGF. Results are expressed as mean ± s.e.m. The statistical significance of the differences between the control and inhibitor-treated rats was evaluated using Student's *t*-test.

* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

Fig. 2 The effect of an angiotensin converting enzyme inhibitor (SQ 14225) or an angiotensin II receptor antagonist (saralasin, P113) or NGF antiserum on NGF-induced pressor responses. Blood pressure, before and after test injections into the right jugular vein, was recorded with a strain-gauge transducer from the carotid artery of the rat anaesthetised with urethane (1 g per kg body weight intraperitoneally) and ganglion-blockaded (50 mg hexamethonium per kg body weight subcutaneously, with additional amounts as required). Inhibitors were injected 5 min before NGF administration. Responsiveness to AII was always confirmed in animals which had failed to respond to NGF after pretreatment with SQ 14225 or NGF antiserum (8.3 μ l diluted to 100 μ l; from Dr G. Guroff).

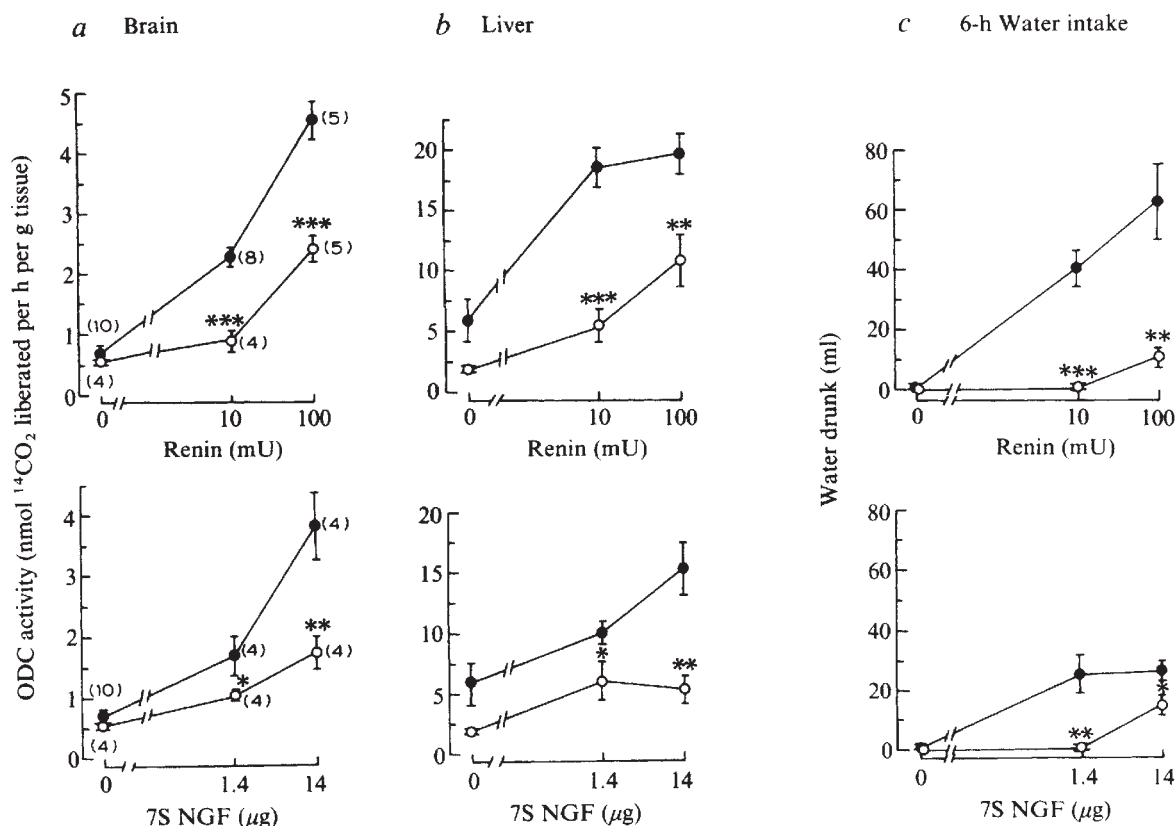
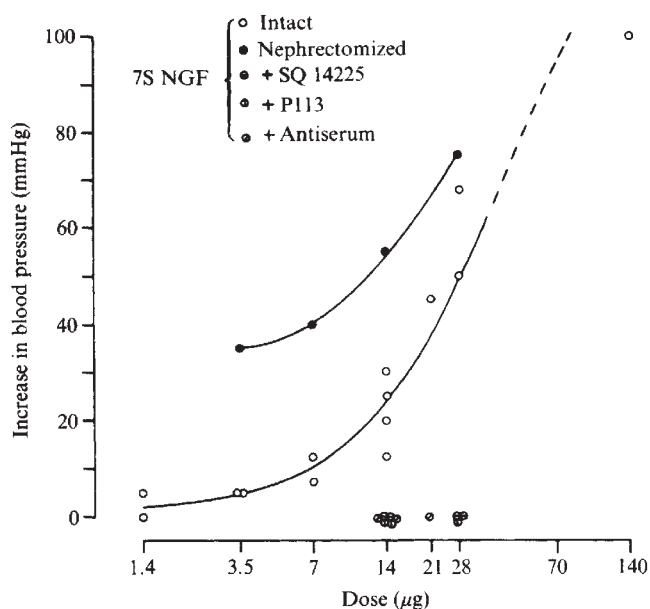


Fig. 3 The effect of a converting enzyme inhibitor (SQ 14225) on renin- and NGF-induced increases in ornithine decarboxylase activity in brain and liver and water intake in the same animals. Rats were given intraventricular injections¹⁷ of either 7 μ l or 0.9% NaCl or 7 μ l 0.9% NaCl containing 35 μ g SQ 14225, followed 2 min later by 5 μ l injections of 0.9% NaCl, renin or 7S NGF. Six hours later, water intakes were recorded, the rats were killed and their brains and livers removed and frozen on solid CO₂. The activity of ornithine decarboxylase (ODC) in these tissues was measured essentially as described before^{17,18} (mean $\pm$ s.e.m. is indicated with the number of animals in parentheses). The values for the control (●) and inhibitor-treated (○) animals were compared statistically as before. * $P < 0.05$; ** $P < 0.01$.

A further question which has not been resolved is whether a renin-like enzyme should be regarded as an integral component of mouse submandibular gland NGF complexes. Isorenin and NGF are stored together in the same cells of the mouse submandibular gland²⁴, are closely associated physically²⁴, show the same sexual dimorphism and the same changes with age^{25,26}, and are both secreted in response to α -adrenergic stimulation^{27,28}.

In conclusion, experiments with inhibitors of the renin-angiotensin system demonstrate that several potent behavioural and biological effects of NGF preparations depend on the generation of AII by a renin-like enzyme in NGF. Therefore, unless renin-free preparations of NGF are used, antagonists of the renin-angiotensin system such as saralasin and SQ 14225 must be used in testing for putative NGF effects, particularly when a biological effect attributed to NGF is known to be produced by renin or AII.

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Epidermal growth factor requirement for development of cultured mammary gland

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The mouse mammary gland in serum-free whole organ culture can be manipulated hormonally to undergo one complete physiological cycle consisting of lobuloalveolar development¹, functional differentiation and regression², mimicking processes that occur *in vivo*. A second cycle has not previously been achieved *in vitro*. The present study has identified a specific requirement for epidermal growth factor (EGF) in the morphological development of mammary lobuloalveoli, allowing two complete cycles of development and regression in culture.

Whole mammary glands of BALB/c mice were cultured for 9 days in serum-free development medium containing insulin (I), prolactin (P), aldosterone (A) and hydrocortisone (H) (Fig. 1 legend)^{3,4}. At day 9, the glands contain fully developed lobuloalveoli^{2,3} and casein⁵. When such glands are maintained in hormone-deficient regression medium (insulin as the only added hormone) for an additional 15 days (days 9-24), the lobuloalveoli involute (regress)^{2,3}. Such regressed glands did not undergo a second development on return to the above IPAH medium (medium containing insulin, prolactin, aldosterone and hydrocortisone) for an additional 9 days (Fig. 1, Table 1). They were presumably depleted of necessary growth factor(s).

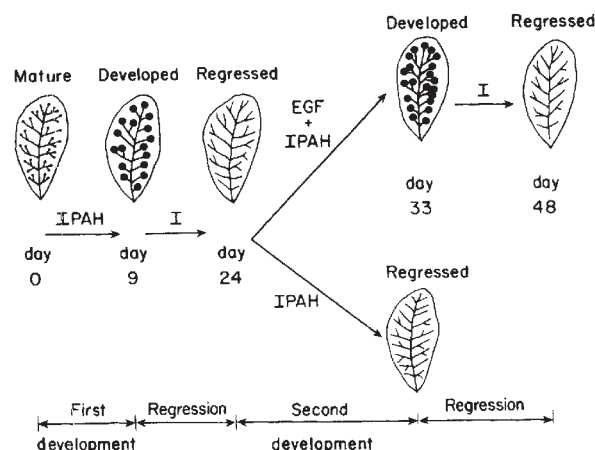


Fig. 1 Two cycles of development and regression of mouse mammary glands in whole organ culture. The serum-free culture system has been described^{3,4,12}. Female BALB/c mice, 3-4 weeks old, were primed with 9 daily injections of β -oestradiol (1 μ g) and progesterone (1 mg). On the day following the last injection, mice were killed and their entire second thoracic mammary glands were excised and floated on Dacron rafts. The serum-free development medium consisted of Waymouth MB752/1 medium (1 ml per gland) supplemented with 350 μ g ml⁻¹ L-glutamine, 35 μ g ml⁻¹ penicillin G, and 5 μ g ml⁻¹ of each of the hormones, insulin (I), prolactin (P), aldosterone (A) and hydrocortisone (H). Medium was changed every 2 to 3 days. Glands cultured in the development medium for 9 days (days 0-9) underwent first lobuloalveolar development^{1-3,12}. Exposure of these glands to regression medium containing insulin (5 μ g ml⁻¹) as the only added hormone for additional 15 days (days 9-24) caused first regression of the mammary lobuloalveoli^{2,3,12}. Glands were then incubated in development medium with EGF for another 9 days (days 24-33), resulting in second development of the mammary glands. Second-developed mammary glands were then regressed in the regression medium for an additional 15 days (days 33-48). Glands were fixed, stained, coded³ and evaluated as in Table 1.

We have found that the polypeptide, EGF, a purified mitogenic protein of molecular weight 6,100 (ref. 6), specifically induces a second development in previously regressed whole mammary glands. The presence of EGF in combination with IPAH for 9 days (days 24-33) elicited this effect in more than 71% of the glands in the absence of serum (Fig. 1, Table 1). The development was verified by microscopic examinations of the whole organ (Fig. 2) and at the cellular level. This positive response was in marked contrast to the refractoriness of the glands to the polypeptides, nerve growth factor, fibroblast growth factor, and multiplication stimulating activity factor, each in the presence of IPAH. Supplementations of IPAH with fetal bovine serum (FBS), growth hormone, β -oestradiol, and progesterone singly or in combinations were also ineffective. The addition of growth hormone to IPAH and EGF resulted in no additional development, and the combination of growth hormone, FBS and IPAH actually inhibited the second development-promoting activity of EGF.

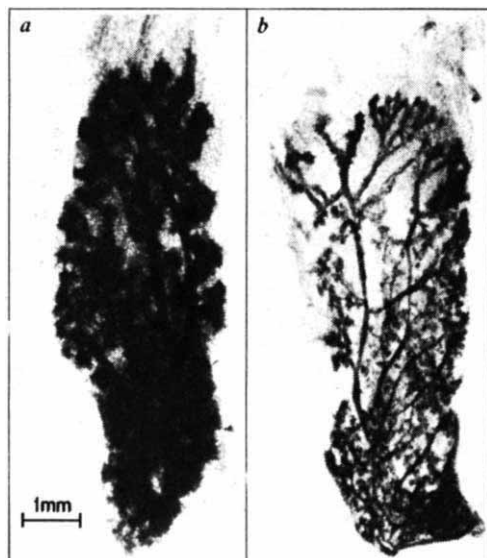


Fig. 2 Second lobuloalveolar development of mouse mammary gland in whole organ culture. Entire mammary glands were cultured for 24 days according to the protocol described in Fig. 1 legend. Glands were cultured for an additional 9 days (days 24–33) in the IPAH medium with or without 10 nM EGF. Glands were then processed as described in Fig. 1 legend and Table 1. *a*, Whole mammary gland showing second-developed lobuloalveoli after incubation in the medium containing EGF. *b*, Control gland similarly incubated in the medium without EGF. The epithelial cells are primarily ductal, and alveoli are absent.

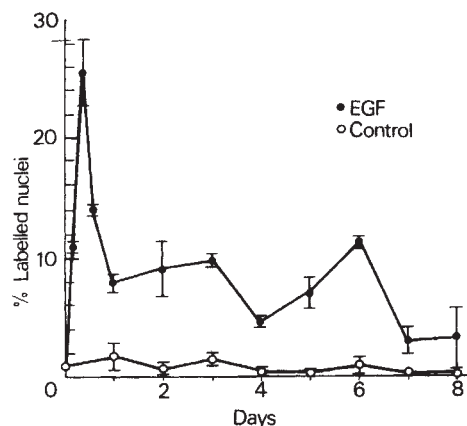


Fig. 3 ^3H -thymidine incorporation in mammary epithelial cell nuclei during second development of mouse mammary glands in whole organ culture. After first development (days 0–9) and regression (days 9–24), as in Fig. 1 legend, the mammary glands were placed in serum-free medium containing insulin, prolactin, aldosterone and hydrocortisone ($5 \mu\text{g ml}^{-1}$ each) in the presence (●) or absence (○) of 10 nM EGF. The presence of mammary fat surrounding the epithelial cells within the fat pad apparently necessitated the uses of relatively high levels of radioactivity and long exposure time. Glands were pulsed for 1 h with $5 \mu\text{Ci ml}^{-1}$ ^3H -thymidine (128Ci mmol^{-1} ; NEN) at the different times, and then were fixed³. Autoradiographs of 5- μm histological sections were prepared by dipping the slides in Kodak NTB-2 emulsion. The coated slides were placed in toluene with scintillator (4 g PPO, 0.05 g POPOP per litre), and exposed for 10 days in the dark at -60°C . Slides were developed²¹, stained with haematoxylin and eosin, and mounted for examination. Epithelial cell nuclei with more than 5 silver grains were scored as positive. A minimum of 500 nuclei were counted from each section. Results from 4 sections are expressed as average numbers of labelled nuclei $\pm$ standard deviations (vertical limits).

The activity of EGF was concentration dependent. Significant activity was absent at 1 nM or less, but was maximal at 10 nM and 100 nM, inducing second development in 70 and 75% of the glands, respectively ($P < 0.0001$). The latter concentrations of EGF approximate those present in mammalian tissues⁷.

The second development induced by EGF and IPAH apparently stimulated the mammary epithelial cells to undergo synthesis of DNA over the course of 1 week. As determined by ^3H -thymidine incorporated into cell nuclei, a peak of DNA synthesis occurred in 25% of the mammary epithelial cells at 8 h after addition of EGF, with perhaps a second peak at day 3, and a third at day 6 (Fig. 3). This periodicity is compatible with a cell generation of 3 days.

Glands that underwent second development in EGF (10 nM) and IPAH (each $5 \mu\text{g ml}^{-1}$) regressed at the end of 15 days (days 33–48) in medium containing insulin as the only added hormone (regression medium) (Fig. 1). A third development was not induced in the presence of EGF and IPAH.

Table 1 Specific induction of second mammary development by epidermal growth factor in whole organ culture

Additions to IPAH	Concentration	No. of glands	>50% Alveoli developed	
			Glands	% Glands
None	—	26	2	8
EGF	10 nM	28	20	71*
NGF	10 ng ml ⁻¹	28	4	14
FGF	10 ng ml ⁻¹	26	1	4
MSA	10 ng ml ⁻¹	28	3	11
FBS	10%	9	0	0
G	$5 \mu\text{g ml}^{-1}$	21	2	10
E + Prg	1 ng ml ⁻¹ , 1 $\mu\text{g ml}^{-1}$	34	2	6
EGF + G	10 nM, $5 \mu\text{g ml}^{-1}$	16	8	50*
EGF + G + FBS	10 nM, $5 \mu\text{g ml}^{-1}$, 10%	18	3	17

Mouse mammary glands in serum-free whole organ culture were induced to undergo first development (days 0–9) and then regression (days 9–24), as in the Fig. 1 legend. With the aim of achieving a second development, the fully regressed glands were incubated for another 9 days (days 24–33) in serum-free development medium containing IPAH (each at $5 \mu\text{g ml}^{-1}$) and the listed additions. Epidermal growth factor (purified from male mouse submaxillary glands), nerve growth factor (NGF), fibroblast growth factor (FGF), multiplication stimulation activity factor (MSA) (all Collaborative Research), fetal bovine serum (FBS; Gibco), growth hormone (G; Miles), β -oestradiol and progesterone (E, Prg; Sigma) were present at the stated concentrations. At day 33, the organs were coded, fixed and stained³. The relative extents of development were scored on the basis of the percentage of developed lobuloalveoli in individual glands, 0 representing no development and 100% full development. The percents of the numbers of >50% developed glands are indicated.

* Significant second development according to Fisher's 2×2 exact test.

In contrast to its second development-promoting activity, EGF added alone or in combination with several steroid or polypeptide hormones induced little or no first development of alveoli. The hormones P, I, IA, IH, IAH, AH, each at $5 \mu\text{g ml}^{-1}$, either alone or with EGF (10 nM), did not promote first development. However, the two combinations, IP with EGF, and PAH with EGF, did elicit moderate activities compared to IPAH without EGF (first development medium).

The polypeptide growth factor, EGF, may have a necessary and specific role in the physiological actions of the mammary gland. EGF acting with the hormones IPAH enables the mouse mammary gland to undergo a second development from the fully regressed state in serum-free culture. A stimulation of DNA synthesis seems to be involved. The requirement for EGF

is specific. The activity of EGF is maximal at physiological concentration (10 nM)⁷. Presumably endogenous stores of EGF operate in first development, as exogenous EGF does in second development. The mouse mammary gland can thus be manipulated to undergo two complete cycles of mammary gland development and regression in whole organ culture (Fig. 1).

EGF has been reported to induce mitogenesis⁸⁻¹⁰, DNA synthesis⁸⁻¹¹ and RNA synthesis⁸ in cultured fragments or dissociated cells of mammary glands. Starting with fragments of mammary glands already partly developed *in vivo* during pregnancy, additional development was induced in culture in response to EGF with insulin or hydrocortisone⁸. The present demonstration of the ability of EGF to promote a second development of mammary gland from the fully involuted state, permitting two cycles of development and regression *in vitro*, considerably extends knowledge of the importance of EGF in the regulation of mammary gland development.

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Transformation of mouse mammary glands by chemical or carcinogens in whole organ culture is manifest as a persistence of developed lobuloalveoli in hormone-deficient medium (insulin as the only added hormone). The transformed glands, which are dysplastic, metaplastic^{3,12} and oncogenic¹⁴ have escaped from the growth controls normally imposed by prolactin and specific steroids^{3,14}. The finding of an EGF role in mammary gland development and other observations^{12,15-20} raise the question whether a deregulation of the EGF system may mediate the loss of controls of lobuloalveolar development that characterizes the mammary gland transformation by chemical carcinogens.

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Monkey pituitary oestrogen receptors and the biphasic action of oestradiol on gonadotropin secretion

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Evidence has been advanced in favour of the hypothesis that, in the rhesus monkey, the negative feedback as well as the stimulatory or so-called 'positive feedback' action of oestrogen on gonadotropin secretion is at the level of the pituitary gland¹⁻³. The cellular basis for this biphasic action of oestrogen on the gonadotroph is not understood. In oestrogen target tissues the elicitation of physiological responses is generally associated with the binding of oestrogen to cytoplasmic receptor proteins which are subsequently translocated to the nucleus. Such receptors have been characterized in the anterior pituitary of the rat⁴⁻⁶. In the ovariectomized rhesus monkey infused with ³H-oestradiol, autoradiography⁷ and parallel cell fractionation⁸ have demonstrated the accumulation of ³H-oestradiol by the nuclei of a significant number of pituitary cells. We describe here some of the properties of these putative oestrogen receptors in the anterior pituitary of the monkey and observe that their distribution between cytoplasmic and nuclear compartments is constant in response to sustained elevations in serum oestrogen concentration which produce a biphasic pattern of circulating gonadotropins.

Ten ovariectomized rhesus monkeys which had responded to a trial injection of oestradiol benzoate (30 µg kg⁻¹) by manifesting a sharp decline in circulating LH followed by an LH surge were chosen for this study. Three animals received no further treatment and were kept as controls. Silastic capsules (4 cm long, 3.35 mm internal diameter, 4.65 mm external diameter, 7-9 per

animal) filled with crystalline 17β-oestradiol⁹ were implanted subcutaneously in the other seven animals under ketamine hydrochloride anaesthesia. These implants produced constant peripheral oestradiol concentrations of 300-500 pg per ml serum. The oestradiol-treated animals were killed at the following times: 12-24 h (at the time of negative feedback inhibition of gonadotropin secretion), 44-48 h (at the height of the gonadotropin surge), and 96 h (after the gonadotropin surge). Each animal was anaesthetized with sodium pentobarbital (30 mg kg⁻¹ intravenously), and cannulae were inserted into the carotid arteries. The calvarium was removed, the animal was swiftly decapitated, and the head was perfused with 400 ml ice-cold 0.9% NaCl to remove all traces of blood. The anterior pituitary was dissected free from the posterior pituitary gland, weighed, and processed for measurement of cytoplasmic or nuclear oestrogen receptor content (see figure legends for details).

Oestrogen receptors labelled with ³H-oestradiol in the cytosol (soluble cytoplasmic fraction) from the anterior pituitary of one untreated control animal were found to sediment at approximately 7S in low ionic strength sucrose gradients (Fig. 1). The 7S peak was completely abolished by addition of a 100-fold excess of unlabelled diethylstilboestrol (DES). There was no indication of any contamination of the cytosol fraction by sex hormone binding globulin (SHBG) from the serum¹⁰, as the serum ³H-

Table 1 Pituitary gonadotropin content at various times after implantation of oestradiol-containing Silastic capsules in ovariectomized rhesus monkeys

Oestradiol treatment		Pituitary gonadotropin content			
		LH		FSH	
		(µg per gland)	(ng per mg wet weight)	(µg per gland)	(ng per mg wet weight)
Untreated	(2)	55.8 ± 0.7	900 ± 128	65.6 ± 11.4	1,033 ± 20
12-24 h E ₂	(3)	85.2 ± 8.2	1,370 ± 27	137.3 ± 30.8	2,166 ± 375
44-48 h E ₂	(3)	78.6 ± 31.3	1,349 ± 315	59.7 ± 19.6	1,052 ± 210
96 h E ₂	(1)	26.9	782	<5.5	<100

Rhesus pituitary LH and FSH were measured by specific radioimmunoassays^{22,23}. Number of determinations are in parentheses. Results are expressed as mean ± s.e.m.

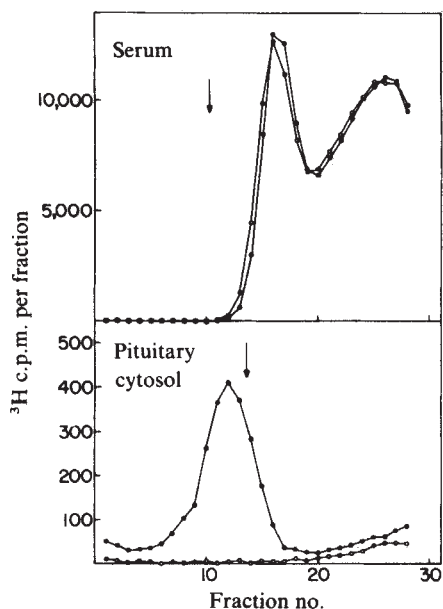


Fig. 1 Sucrose gradient centrifugation of bound ^3H -oestradiol in monkey serum or anterior pituitary cytosol. The anterior pituitary (62 mg) from an untreated ovariectomized monkey was homogenized in 20 vol TED (10 mM Tris-HCl, pH 7.4 at 25 °C, 1.5 mM EDTA, 1 mM dithiothreitol) as described previously¹⁹. Cytosol was prepared by centrifugation of the homogenate for 1 h at 189,000 g. Blood was collected from the same animal by femoral venipuncture and allowed to clot at 4 °C overnight. Serum was separated by centrifugation and diluted 1:10 with TED. Cytosol or diluted serum was incubated for 2 h at 0 °C with 10 nM [2,4,6,7- ^3H]17 β -oestradiol (NEN, 102.0 Ci mmol⁻¹) in the absence (●) or presence (○) of 1 μM diethylstilboestrol (DES). Unbound ^3H -oestradiol was removed from the cytosol fraction by treatment with dextran-charcoal (1/8 vol. 1.0% Dextran T 70, 10% Norit A in TED); samples were vortexed for 10 s, kept in ice for 20 min and centrifuged at 2,075 g for 15 min. Aliquots (0.25 ml) of serum or charcoal-treated cytosol incubates were layered on 5 to 20% linear sucrose gradients in TE buffer (10 mM Tris-HCl, pH 7.4 at 25 °C, 1.5 mM EDTA) and centrifuged in an SW 50.1 rotor at 215,000 g for 14.5 h (serum) or 16 h (cytosol). Eleven-drop fractions were collected directly into scintillation vials and counted. The arrow indicates the position of bacterial alkaline phosphatase added as an internal sedimentation marker (6.2S, see ref. 18). Direction of sedimentation was from right to left.

oestradiol-SHBG complex sedimented at 4S, and binding of ^3H -oestradiol was unaffected by the presence of excess unlabelled DES (Fig. 1).

Scatchard analysis¹¹ of equilibrium binding data obtained by incubating aliquots of pituitary cytosol from two untreated ovariectomized animals with various concentrations of ^3H -oestradiol (0.2–10 nM) in the absence or presence of 1 μM DES indicated the existence of a single class of high affinity, limited capacity binding sites (see Fig. 2 for a representative Scatchard plot). The dissociation constants (K_D 's) obtained from these two experiments were 0.70 and 0.73 nM, and the concentrations of binding sites were 50 and 80 fmol per mg cytosol protein. Very low levels of oestrogen receptors were localized in the nuclear fraction (<0.3 fmol per pituitary) (Fig. 3).

Implantation of Silastic capsules containing oestradiol in ovariectomized monkeys led to depletion of cytoplasmic receptors to 10–20% of the levels found in untreated controls and concomitant accumulation of oestrogen receptors in the nucleus (Fig. 3). The concentrations of cytosol and nuclear receptors were constant during the period of oestrogen treatment. The loss of receptors from the cytoplasm could be accounted for by their presence in the nucleus. The total number of oestrogen receptors in the anterior pituitary were 63.4 (0 h), 62.3 (12–24 h), 64.5 (44–48 h) and 73.1 (96 h) fmol per pituitary when 5 nM ^3H -oestradiol was used for cytosol binding and 72.7 (0 h), 61.4 (12–24 h), 63.6 (44–48 h) and 71.2 (96 h) fmol per pituitary when 10 nM ^3H -oestradiol was used. As a result of this oestrogen stimulus, circulating luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels declined at 12–24 h, peaked at approximately 44–48 h, and decreased again to low levels at 96 h (Fig. 3).

The time course of pituitary gonadotropin concentrations from these animals is shown in Table 1. Pituitary LH and FSH content both seemed to increase during the initial 24 h of oestradiol treatment and decrease again following the gonadotropin surge (96 h). A similar pattern of pituitary gonadotropin content is observed during the menstrual cycle of the rhesus monkey with an accumulation of LH and FSH before the midcycle gonadotropin surge followed by substantial depletion several days later¹². These results are in agreement with the changes in pituitary LH content observed during the rat oestrous cycle¹³ or during a simulated preovulatory LH surge in the rat¹⁴. We do not know at present whether the oestrogen-induced changes in pituitary gonadotropin content in the monkey are due to effects on synthesis, packaging, and/or release of LH and FSH; however, the effectiveness of oestradiol in regulating these processes is dependent on the prior exposure of the pituitary gland to permissive amounts of the hypothalamic gonadotropin-releasing hormone (GnRH)¹⁵. Note that depletion of pituitary gonadotropin stores with prolonged oestradiol treatment is presumably progressive: 2 years after placement of oestradiol implants in two intact female rhesus monkeys, pituitary LH and FSH were reduced to 2–3 ng per mg wet weight (W. D. Peckham, personal communication).

We have demonstrated that the anterior pituitary gland of the ovariectomized rhesus monkey contains cytoplasmic oestrogen receptors with properties similar to those found in rodent anterior pituitary tissue^{4–6}. We assume that oestrogen receptors are present in rhesus pituitary gonadotrophs because of the profound effects of oestradiol on both the morphology¹⁶ and secretory response of these cells. The presence of oestrogen receptors in other pituitary cell types cannot be excluded, however, as ^3H -oestradiol has been localized by autoradiography in the nucleus of acidophils as well as basophils in the rhesus pituitary gland⁷.

A sustained increment in circulating oestradiol leads to depletion of cytoplasmic receptors and their accumulation in the nucleus where they remain as long as serum oestradiol levels remain elevated. This same oestradiol stimulus results in a decline in gonadotropin secretion followed by a massive discharge of LH and FSH. Although it may not seem surprising that the interaction of oestradiol with the monkey pituitary conforms to the general model for oestrogen action in other target cells, the dynamics of oestrogen receptor populations have not been

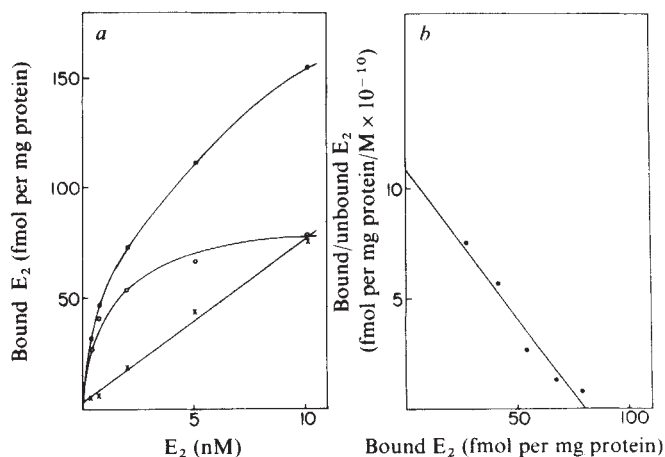


Fig. 2 Binding of ^3H -oestradiol (E_2) by monkey anterior pituitary cytosol as a function of ^3H -oestradiol concentration (a) or as a Scatchard plot (b). A hemi-anterior pituitary (40 mg) from an ovariectomized untreated monkey was homogenized in 40 vol TED, and cytosol was prepared as described in Fig. 1 legend. Aliquots of cytosol were incubated with various concentrations of ^3H -oestradiol (0.2–4.5 nM) in the absence or presence of 1 μM DES (total vol 100 μl). Incubation times, charcoal treatment to separate bound and unbound ^3H -oestradiol, and radioactivity measurements are given in Fig. 3 legend. a, Specific binding (○) represents the difference between total binding measured in the absence of excess DES (●) and nonspecific binding (×) measured in its presence. Cytosol protein was determined by the method of Lowry *et al.*¹⁸ using crystallized bovine serum albumin as standard.

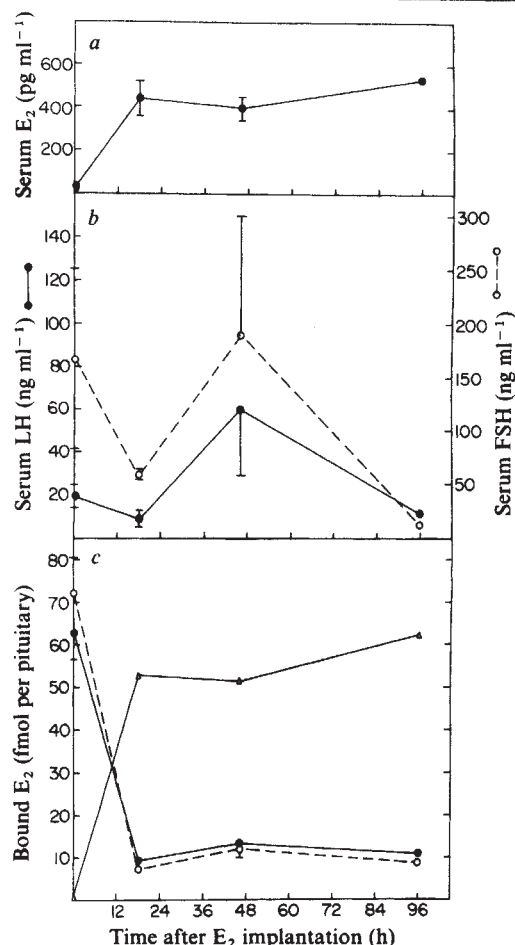


Fig. 3 Content of specific oestrogen binding sites in cytosol and nuclear fractions from the anterior pituitary of ovariectomized rhesus monkeys and circulating gonadotropin concentrations as a function of time after the implantation of Silastic capsules containing crystalline 17β -oestradiol. Placement of implants and killing of animals are described in the text. Previously reported radioimmunoassays were used to measure serum oestradiol^{20,21} (a), LH²² and FSH²³ (b) in blood samples taken immediately before killing. Specific binding of ^3H -oestradiol to cytoplasmic and nuclear receptors was determined by exchange assays (c). Data collected at times of negative feedback (12–24 h) or of positive feedback (44–48 h) have been plotted as the means of these times, 18 h and 46 h, respectively. Cytosol was prepared as described in Fig. 1 except that anterior pituitary tissue was homogenized in 40 vol TED. Cytosol fractions, treated with dextran-charcoal to remove any possible contaminating endogenous oestradiol, were incubated with saturating concentrations of ^3H -oestradiol, 5 (●) or 10 (○) nM, in the absence or presence of $1\ \mu\text{M}$ DES for 2 h at 0°C to fill any unoccupied receptor sites followed by 16 h at 24°C (to allow exchange of ^3H -oestradiol with receptor sites filled with endogenous oestradiol)^{24,25}. Total volume of incubation mixtures was $100\ \mu\text{l}$. After incubation, samples were placed in ice for 30 min and unbound ^3H -oestradiol was removed by addition of $25\ \mu\text{l}$ 5% Norit A–0.5% Dextran T70 as in Fig. 1. Aliquots ($50\ \mu\text{l}$) of the charcoal-treated supernatants were counted in 5 ml Econofluor in a Packard scintillation spectrometer with an efficiency of 33%. The receptor measurements illustrated represent single determinations (46 h, 5 nM ^3H -oestradiol; 18 h 10 nM ^3H -oestradiol; 96 h, 5 and 10 nM ^3H -oestradiol) or the mean $\pm$ s.e.m. of 2 (18 h, 5 nM ^3H -oestradiol; 46 h, 10 nM ^3H -oestradiol) or 3 (0 h, 5 or 10 nM ^3H -oestradiol) determinations. For measurement of nuclear receptors, anterior pituitary tissue was homogenized and nuclei isolated by the method of Zigmund and McEwen²⁶. Nuclear oestrogen receptors were assayed as described by Roy and McEwen²⁷. Briefly, receptors were salt-extracted from nuclei swollen in hypotonic buffer and incubated with 10 nM ^3H -oestradiol, with or without $1\ \mu\text{M}$ DES, for 16 h at 25°C in a total volume of $125\ \mu\text{l}$. Aliquots ($100\ \mu\text{l}$) of the incubation mixtures were applied to $6 \times 50\ \text{mm}$ Sephadex LH-20 columns equilibrated with TEK-0.4 (10 mM Tris-HCl, pH 7.4, 1.5 mM EDTA, 0.4 M KCl) and bound ^3H -oestradiol was eluted into scintillation vials with 1 ml of the same buffer. Econofluor (10) was added, and after vigorous shaking, samples were counted as above with an efficiency of 38%. Nuclei were dissolved in 0.3 M KOH, and DNA contents were measured by the procedure of Burton²⁸. Each data point (Δ) represents a single determination. The corresponding values in fmol per mg DNA are 329 (18 h), 383 (46 h), and 277 (96 h).

examined previously in conditions where this steroid exerts both an inhibitory and a stimulatory effect on the same process, presumably in the same cell type. Thus, any hypothesis for the mechanism of action of oestradiol in the sequential inhibition and stimulation of glycoprotein hormone secretion must appreciate that this biphasic effect of the steroid on the anterior pituitary is manifested in the face of an unchanging distribution of cytoplasmic and nuclear oestrogen receptors.

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Glucocorticoids increase osteoblast-like bone cell response to $1,25(\text{OH})_2\text{D}_3$

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Recent reports indicate that some hormones may regulate the binding of, and subsequent response to, other hormones by their target tissue^{1–6}. The adrenal glucocorticoids are prominent among these modulating hormones^{3–6}. Glucocorticoids have been shown to enhance bone cell sensitivity to parathyroid hormone (PTH)^{6,7} *in vitro* and this in turn has permitted PTH-induced effects to be measured at physiological doses of PTH for the first time in isolated osteoblast-like (OB) and osteoclast-like (OC) cells. It is unknown whether these findings represent a specific interaction between glucocorticoids and PTH⁶ or indicate a general role for glucocorticoids in the development and/or maintenance of bone cell differentiation, of which hormonal responsiveness would be one expression. In the event of a general glucocorticoid effect on cell differentiation, increased responsiveness to other bone resorbing hormones should also be observed. We have therefore examined whether glucocorticoids enhance the sensitivity of bone cells to a steroid hormone, $1,25$ dihydroxycholecalciferol ($1,25(\text{OH})_2\text{D}_3$), and we report here that they do.

1,25(OH)₂D₃ can induce resorption of bone *in vivo*⁸⁻¹⁰ and *in vitro*¹¹. The biochemical changes induced by 1,25(OH)₂D₃ are very similar to those initiated by PTH—in both cases bone mineral and organic matrix loss is observed. PTH also stimulates hyaluronic acid synthesis^{12,13} and lysosomal enzyme release^{14,15} and inhibits collagen formation^{16,17}, alkaline phosphatase activity¹⁸ and citrate decarboxylation¹⁹. Some of these metabolic events that accompany PTH-induced bone resorption have been reproduced in OC or OB bone cells exposed to pharmacological doses of 1,25(OH)₂D₃ (ref. 20). However, physiological doses of 1,25(OH)₂D₃ were without effect in these cells.

In previous studies low doses of glucocorticoids decreased basal osteoclastic but not osteoblastic activities. As a result the major enhancement of PTH responsiveness by glucocorticoids occurred in OB cells⁷. For this reason, these studies on the relationship between glucocorticoids and 1,25(OH)₂D₃ were carried out on OB bone cells. Inhibition of OB cell citrate decarboxylation by a submaximal dose of 1,25(OH)₂D₃ (100 pg ml⁻¹) was measured alone or in combination with various doses of glucocorticoids (Fig. 1a). At prednisolone levels below 10⁻¹⁰ M the inhibition of citrate decarboxylation by 1,25(OH)₂D₃ was unchanged. However, at 10⁻⁹–10⁻⁷ M prednisolone, the decrease in citrate decarboxylation due to 1,25(OH)₂D₃ was greater than in cells not treated with glucocorticoid. Prednisolone enhancement of the effects of 1,25(OH)₂D₃ resulted from both a decrease in the absolute amount of citrate decarboxylated in the presence of 1,25(OH)₂D₃ and a small increase in basal citrate decarboxylation. To compare the relative enhancement by different doses of prednisolone the inhibition of citrate decarboxylation by 1,25(OH)₂D₃ is further expressed as a percentage of the appropriate glucocorticoid-treated control (Fig. 1b). Maximum potentiation of 1,25(OH)₂D₃ action was seen with 10⁻⁹ and 10⁻⁸ M prednisolone.

OB cell response to various doses of 1,25(OH)₂D₃ was measured in the absence or presence of a maximally effective dose of prednisolone (Fig. 2). The dose-response curve for 1,25(OH)₂D₃ became shifted to the left in the presence of 10⁻⁹ M prednisolone and 2–10-fold lower doses of 1,25(OH)₂D₃ were required to bring about an established degree of inhibition of citrate decarboxylation. Addition of 10⁻⁹ M prednisolone to the culture medium of OB cells permitted levels of 1,25(OH)₂D₃ as low as 5–25 pg ml⁻¹ (amounts well within normal circulating levels) to be assayed for the first time in this *in vitro* cell culture system. Note that although the absolute sensitivity of the isolated OB cells to

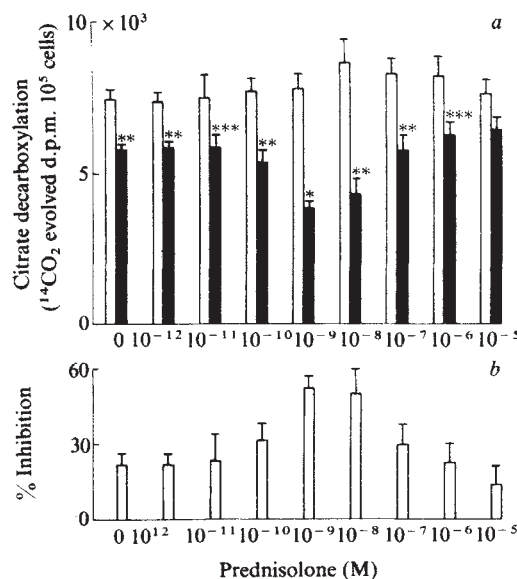


Fig. 1 Effect of various doses of glucocorticoid on OB bone cell response to 1,25(OH)₂D₃. Primary cultures of OB bone cells were prepared as previously described²⁴, subdivided after 5 d and inoculated into glass test tubes (1.0 × 10⁵ cells per tube) containing 1.0 ml (MEM) and 10% fetal calf serum. *a*, Cells were cultured with the indicated doses of prednisolone in the absence (open bars) or presence (closed bars) of 1,25(OH)₂D₃ (100 pg ml⁻¹). After 48 h, citrate decarboxylation was measured by the addition of [6-¹⁴C]citrate (0.2 μCi ml⁻¹) for 2 h. ¹⁴CO₂ was trapped on filter paper fans saturated with Hyamine × 10 as previously described^{19,25}. *b*, Results are expressed as the per cent inhibition seen when 1,25(OH)₂D₃-treated cultures were compared with the appropriate prednisolone-treated control. These values represent the average of four determinations ± s.d., and these experiments were performed five times, with similar results. **P* < 0.005; ***P* < 0.002; ****P* < 0.05.

1,25(OH)₂D₃ varied from preparation to preparation, the relative increased sensitivity in the presence of prednisolone was always maintained.

Increased sensitivity to 1,25(OH)₂D₃ could also be demonstrated when OB cell collagen synthesis was measured in prednisolone-treated cells exposed to 1,25(OH)₂D₃ (Table 1). Inhibition of collagen synthesis by 1,25(OH)₂D₃ increased from

Table 1 Effects of prednisolone and 1,25(OH)₂D₃ on collagen synthesis by OB bone cells

	Collagen (³ H-proline d.p.m. per 10 cells)	Non-collagen protein (³ H-proline d.p.m. per 10 cells)	% Decrease	% Total protein*
Control	5,500 ± 300	6,200 ± 360	—	13.0
Control + 1,25(OH) ₂ D ₃	3,885 ± 350‡	6,000 ± 450	29	10.0
10 ⁻⁴ M Prednisolone	3,930 ± 400	5,300 ± 400	—	11.0
10 ⁻⁴ M Prednisolone + 1,25(OH) ₂ D ₃	3,310 ± 150	4,700 ± 410	16	11.0
10 ⁻⁶ M Prednisolone	5,380 ± 270	6,500 ± 350	—	12.0
10 ⁻⁶ M Prednisolone + 1,25(OH) ₂ D ₃	3,450 ± 190†	5,850 ± 390	36	9.0
10 ⁻⁸ M Prednisolone	5,700 ± 330	6,230 ± 410	—	13.0
10 ⁻⁸ M Prednisolone + 1,25(OH) ₂ D ₃	3,200 ± 210†	6,000 ± 380	44	8.0
10 ⁻¹⁰ M Prednisolone	5,000 ± 400	6,200 ± 350	—	13.0
10 ⁻¹⁰ M Prednisolone + 1,25(OH) ₂ D ₃	3,800 ± 250‡	5,650 ± 430	24	10.0

OB cells were cultured in the presence of 10⁻¹⁰–10⁻⁴ M prednisolone alone, or in combination with 1,25(OH)₂D₃ (125 pg ml⁻¹). After 48 h the growth medium was removed, and the cells were further cultured in minimal essential medium containing 1.0 μCi ml⁻¹ L-[2,3-³H]proline (50 Ci mmol⁻¹) for 24 h. The media and the cells were precipitated *in situ* with 5% trichloroacetic acid (TCA). The precipitate was centrifuged and washed 4 times over a 24-h period with 12 ml ice-cold 5% TCA. The radioactivity rendered acid soluble by heating the cell pellets at 90 °C for 45 min in 1 ml 5% TCA was used as a measure of collagen. Alternatively, the cell layer collagen was rendered acid soluble by digestion with purified bacterial collagenase. The acid-insoluble radioactivity remaining after these procedures (non-collagenous protein) was dissolved by heating at 60 °C for 30 min in 0.5 ml 0.2 M NaOH. Each value represents the mean of triplicate determinations ± s.d.

* Corrected for relative proline enrichment in collagen by a factor of 5.8.

† *P* < 0.005. ‡ *P* < 0.05.

29% in the absence of glucocorticoid to 36% at 10^{-6} M prednisolone, and reached a maximum at 44% at 10^{-8} M prednisolone; no enhancement was seen at 10^{-10} M prednisolone. Glucocorticoid amplifications of the effects of $1,25(\text{OH})_2\text{D}_3$ on collagen and citrate decarboxylation thus occurred over a similar dose range. The additional decrease in collagen formation seen with $1,25(\text{OH})_2\text{D}_3$ acting in the presence of prednisolone was specific as there was no corresponding change induced in the synthesis of non-collagenous protein.

The molecular basis for glucocorticoid amplification of $1,25(\text{OH})_2\text{D}_3$ effects remains to be established. $1,25(\text{OH})_2\text{D}_3$ interacts with target cells by binding to high affinity cytosolic receptors. Such receptors have been identified in fetal rat calvaria²¹ and it was recently reported that glucocorticoids retard the turnover rate of $1,25(\text{OH})_2\text{D}_3$ receptors in bone tissue in culture²².

In the following experiments, the ability of primary cultures of OB cells to accumulate ^3H -labelled $1,25(\text{OH})_2\text{D}_3$ was measured after growth in the presence or absence of glucocorticoids. Figure 3a demonstrates that after exposure to 10^{-9} or 10^{-7} M but not to 10^{-5} M prednisolone, OB cells showed a greater uptake of ^3H - $1,25(\text{OH})_2\text{D}_3$ than untreated controls, with $10^{-9}\text{ M} > 10^{-7}\text{ M} > 10^{-5}\text{ M}$. At 10^{-9} M and 10^{-7} M glucocorticoid the uptake per cell of ^3H - $1,25(\text{OH})_2\text{D}_3$ was increased ~60% and 40%, respectively, whereas no increase was seen at 10^{-5} M. The increase in cellular uptake of ^3H - $1,25(\text{OH})_2\text{D}_3$ thus coincided with the prednisolone dose curve for enhancement of the biological effects of $1,25(\text{OH})_2\text{D}_3$ in OB cells.

The specificity of OB cell uptake of ^3H - $1,25(\text{OH})_2\text{D}_3$ was measured by competition with unlabelled $1,25(\text{OH})_2\text{D}_3$. The addition of unlabelled $1,25(\text{OH})_2\text{D}_3$ to the medium at the time of exposure to ^3H - $1,25(\text{OH})_2\text{D}_3$ led to a decrease in the radioactivity ultimately recovered with the cells. Addition of 0.1, 1.0 or 10.0 ng ml^{-1} unlabelled $1,25(\text{OH})_2\text{D}_3$ resulted in a decrease of 27%, 50% and 72%, respectively (Fig. 3b). Approximately 17% of the cell-associated radioactivity could not be displaced by $1,25(\text{OH})_2\text{D}_3$ levels as high as 1,000 ng ml^{-1} , and must, therefore, be regarded as nonspecific binding. By comparison, even 10 ng ml^{-1} $24,25(\text{OH})_2\text{D}_3$ reduced cell-associated radioactivity by <20% (Fig. 3c). $25(\text{OH})_2\text{D}_3$ at 10 ng ml^{-1} also reduced cell-associated radioactivity by <20% (results not shown).

These results show that OB bone cells accumulate increased amounts of ^3H - $1,25(\text{OH})_2\text{D}_3$ after exposure to low physiological doses of glucocorticoids. Increased accumulation of ^3H - $1,25(\text{OH})_2\text{D}_3$ by glucocorticoid-treated cells could be due to a

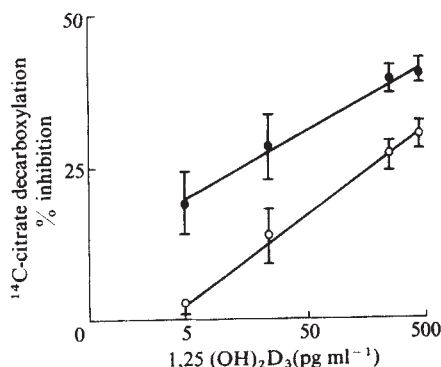


Fig. 2 Amplification of bone cell response to $1,25(\text{OH})_2\text{D}_3$ by glucocorticoid. Subcultured OB bone cells were grown in various concentrations of $1,25(\text{OH})_2\text{D}_3$ alone (○) or further supplemented with 10^{-9} M prednisolone (●). After 48 h citrate decarboxylation was measured as the amount of ^{14}C evolved from $[6-^{14}\text{C}]$ citrate ($0.2 \mu\text{Ci ml}^{-1}$) added to each tube for the final 2 h. Results are expressed as the per cent decrease obtained when $1,25(\text{OH})_2\text{D}_3$ -treated cultures were compared with the appropriate control and each point represents the average of four replicate samples $\pm$ s.d. These experiments were performed six times.

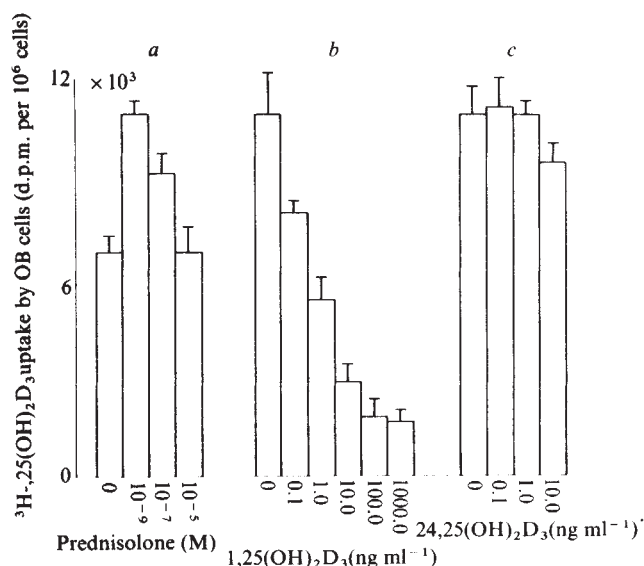


Fig. 3 Effects of prednisolone on the uptake of ^3H - $1,25(\text{OH})_2\text{D}_3$ by OB cells. Primary cultures of 2.0 – 2.5×10^6 cells were exposed to 0, 10^{-5} , 10^{-7} or 10^{-9} M prednisolone for 48 h. Cell monolayers were then rinsed two times with MEM. *a*, Cells were further incubated with 3 ml MEM containing $30,000 \text{ pmol ml}^{-1}$ of $[23,24(n-^3\text{H})]1,25(\text{OH})_2\text{D}_3$ (94 Ci mmol^{-1}) (Amersham). After 60 min at 37°C the medium was aspirated off, and the monolayers were rinsed in phosphate-buffered saline (PBS). The cells were recovered from the tissue culture flask by suspension in calcium-magnesium-free Tyrode's solution containing 0.005 M EDTA, pH 7.0 (EDTA/Tyrodes). Cells were pelleted by centrifugation at 600g for 2 min, then rinsed five times with 10 ml PBS. (At this stage the total radioactivity in the wash was <20% of that associated with the cells.) The cell pellet was then resuspended in 2 ml EDTA/Tyrodes to obtain single cells and 0.025 ml was removed for cell counting in a haemocytometer. The remaining cells were collected on a Millipore filter, which was further rinsed with 10 ml PBS, dissolved in methyl Cellosolve, mixed with aqueous scintillant, and counted in a Searle liquid scintillation counter. *b*, *c*, ^3H - $1,25(\text{OH})_2\text{D}_3$ uptake (by OB cells) was measured as described above except either $1,25(\text{OH})_2\text{D}_3$ (*b*) or $24,25(\text{OH})_2\text{D}_3$ (*c*) was added to the cells at the same time as ^3H - $1,25(\text{OH})_2\text{D}_3$. These measurements represent the average of three determinations $\pm$ s.d. and these experiments were performed four times.

variety of reasons including: (1) an increase in total cell protein; (2) increased cell permeability to steroids including $1,25(\text{OH})_2\text{D}_3$; or (3) a specific increase in the numbers or affinity of the receptors for $1,25(\text{OH})_2\text{D}_3$. As incorporation of ^3H -proline (Table 1) and ^3H -leucine⁷ into acid precipitable radioactivity was not increased in cells cultured in glucocorticoids, the first possibility seems unlikely. Furthermore, the relative inability of $24,25(\text{OH})_2\text{D}_3$ and $25(\text{OH})_2\text{D}_3$ to compete with ^3H - $1,25(\text{OH})_2\text{D}_3$ and the ability of $1,25(\text{OH})_2\text{D}_3$ to decrease cell-associated ^3H - $1,25(\text{OH})_2\text{D}_3$ suggest that the increased accumulation of ^3H - $1,25(\text{OH})_2\text{D}_3$ by glucocorticoid-treated cells was not due to a general increase in cell permeability, but rather was a phenomenon specific to $1,25(\text{OH})_2\text{D}_3$.

The enhancement of $1,25(\text{OH})_2\text{D}_3$ effects on OB bone cells by glucocorticoids is of interest from the molecular as well as physiological point of view. These results imply that glucocorticoids *in vivo* permit bone tissue to respond to small increases in $1,25(\text{OH})_2\text{D}_3$. In addition, the permissive role of glucocorticoids for $1,25(\text{OH})_2\text{D}_3$ parallels that previously described for PTH although the mechanism of action of these two hormones differ at the onset. Glucocorticoids must therefore control a complex set of cellular reactions which can result in amplification of response to at least two classes of bone resorbing hormones, one of which activates adenylate cyclase via cell surface receptors,

the other of which binds to intracellular cytosolic receptors. If hormone sensitivity can be used as a marker for cell specialisation, then glucocorticoids can be further said to enhance the expression of the differentiated phenotype in bone cells, and *in vivo* may exert some control on bone cell development and maintenance.

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Allotype-linked genetic control of a polymorphic V_H framework determinant on mouse T-helper cell receptors

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The immune system confronts antigens through the variable domains of antigen receptors on lymphocytes. The biochemistry of these antigen receptors is fundamental to the understanding of the immune system. Whilst B cells use immunoglobulins (Ig) as antigen receptors, T cells use unknown molecules which appear to differ from Ig except for the shared variable region of the Ig heavy chain (V_H). The notion that T-cell receptors consist, at least in part, of immunoglobulin V_H regions stems largely from experiments using anti-idiotypic antibodies¹⁻³. Much serological and genetic evidence has meanwhile accumulated suggesting that V_H associated idiotype determinants are shared between Ig molecules and the antigen receptors of certain classes of T cells including helper cells, cells proliferating on mixed lymphocyte reaction delayed type hypersensitive reactive cells, and cytotoxic T cells⁴⁻⁷. This pertains to structural elements associated with hypervariable portions of V_H, but data on framework elements of V_H regions of T cells are scarce^{8,9}. We have previously reported that mouse T-cell receptors react with a rabbit antibody to the V_H fragment of MOPC 315 (anti-V_H)¹⁰⁻¹². The V_H framework determinants appeared polymorphic in that the T-helper cells as well as the immunoglobulin heavy (H) chains of a variety of mouse strains reacted equally well, except in the case of strain AKR^{12,13}. We report here the genetic control of the V_H framework determinant whose expression through T-helper cells seems to be governed by at least one gene linked to the Igh allotype linkage group. Thus, this V_H framework determinant behaves like the rabbit V_H region allotypes which are expressed on both immunoglobulin H chains and T-cell receptors^{8,9}. These data suggest the expression of entire V_H regions in T-cell antigen receptors¹⁻³.

The analysis of the reactivity of anti-V_H with T-helper cells involved the *in vitro* responses of spleen cells from mice previously immunized with streptococcus A (ref. 14) to trinitrophenylated group A streptococci (TNP-Strep.A). This T-helper cell-dependent response is inhibited by addition of anti-

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V_H to the culture, whereas inhibition of the T-helper cell-independent response to TNP-Ficoll requires at least a 50-fold greater concentration of anti-V_H (ref. 12). Failure of anti-V_H to react with immunoglobulin molecules and B cells has been reported previously^{10,12,13,15}, suggesting that the inhibition of the TNP-plaque-forming cell (PFC) response to TNP-Strep. A reflects exclusively the inhibition of T-helper cells by anti-V_H (ref. 12).

Preliminary experiments showed that the responses of cells from strains A/J, DBA/2 and BALB/c were strongly inhibited by anti-V_H, whereas those from strain AKR were not (ref. 12, and unpublished data). We therefore crossed strain AKR with DBA/2 and backcrossed the F₁ to AKR. Figure 1 shows the effect of anti-V_H on the *in vitro* responses of spleen cells from mice resulting from this cross to TNP-Strep. A. It is clear that the capacity of cells to be inhibited by anti-V_H is inherited in a dominant fashion. In the backcross, no clear segregation into positive and negative groups can be seen. However, because of the gap between 31% and 38% inhibition, and because all F₁ mice were inhibited more than 38%, we divided the backcross mice into two groups: one group of 15 showed inhibition similar to that of F₁ mice; the other group of 9 were less strongly

Table 1 Association of strong and weak T-helper cell inhibition by anti-V_H and the segregation of the immunoglobulin allotype in (ADR×DBA/2) F₁×AKR backcross mice

	Total No.	Inhibition of help* by anti-V _H		P value†
		<31%	>38%	
(AKR×DBA/2)F ₁				
×AKR total	24	9	15	
H-2 ^k k/k	12	4	8	0.4
d/k	12	5	7	
Thy-1 ⁺ 1/1	10	4	6	0.3
1/2	14	5	9	
Lyt 1 ⁺ 2/2	15	5	10	0.4
1/2	9	4	5	
Lyt 3 ⁺ 1/1	12	4	8	0.4
1/2	12	5	7	
Sex ♂	12	5	7	0.4
♀	12	4	8	
C c/c	9	5	4	0.2
c/C	15	4	11	
Allotype Igh § d/d	10	7	3	<0.005
d/c	14	2	12	

Backcross mice are same as those of Fig. 1.

* See legend to Fig. 1.

† χ^2 Test used.

‡ Determined by microcytotoxicity assay using rabbit complement and lymphocytes from cervical lymph node.

§ Allotype was determined in Ouchterlony analysis using an A/J anti-BALB/c antiserum for Igh^c and a BALB/c anti-A/J antiserum for Igh^d allotype.

|| C. Coat colour.

Table 2 Genetic polymorphism and dominant inheritance of helper cell inhibition by anti- V_H , using partially helper cell depleted spleen cells

Strain		TNP-PFC per 10^6 cells*		% Inhibition
		-a V_H	+a V_H	
AKR	1	223(1.10)	230(1.32)	-3
	2	196(1.12)	192(1.31)	2
	3	201(1.04)	191(1.09)	5
	4	304(1.10)	272(1.14)	12
	5	174(1.09)	166(1.42)	5
	6	94(1.22)	87(1.21)	7
	7	89(1.62)	95(1.44)	-6
	8	104(1.08)	95(1.31)	9
DBA/2	1	422(1.17)	190(1.11)	55
	2	392(1.15)	223(1.14)	43
	3	210(1.14)	92(1.31)	56
	4	312(1.14)	155(1.27)	50
	5	298(1.08)	104(1.31)	65
(AKR $\times$ DBA/2) F_1				
	1	190(1.18)	76(1.09)	60
	2	143(1.10)	63(1.24)	56
	3	198(1.30)	106(1.27)	46
	4	112(1.43)	43(1.71)	62
	5	256(1.20)	122(1.25)	56

* See legend to Fig. 2.

inhibited. These groups were then analysed with respect to seven different genetic markers—H-2, Thy 1, Lyt 1, Lyt 3, sex, coat colour, allotype—segregating in this cross. Table 1 shows that there is no association with any of these markers, except for the Igh allotype with which a significant association is observed. This suggested that this allotype had an important role in the genetic control of V_H expression in T-helper cells.

From these experiments the question arose as to whether the expression of V_H on T cells is exclusively by the Igh allotype controlled or, as would seem more likely from the data in Fig. 1 and Table 1, whether it is controlled by additional genetic factors. We investigated this by improving the quality of the T-helper cell inhibition assay using partially helper cell-depleted spleen cells as responders. This was done by mixing 20% of untreated spleen cells with 80% anti-Thy-1.1 and complement-treated spleen cells of each individual mouse. Because of the limitation of T-cell help in such cultures, smaller quantities of anti- V_H can be used for inhibition, so that the possibility of nonspecific inhibition or of B-cell inhibition is minimized¹².

An analysis of AKR, DBA/2 and (AKR $\times$ DBA/2) F_1 mice by this improved procedure again clearly reveals the genetic polymorphism and dominant inheritance of the V_H determinant

(Table 2). Analysis of an additional 20 (AKR $\times$ DBA/2) $\times$ AKR backcross mice by this typing procedure now reveals a clear segregation into a positive and a negative group and unequivocal linkage to the Igh allotype (Table 3, Fig. 2).

These data support our previous observation that T-helper cells express framework portions of immunoglobulin V_H regions^{11,12}. The significance of these results is that the genetic evidence is independent of the serological data^{11,12,15}, and that both lines of evidence point to the same conclusion. The lack of an influence of H-2 observed in these data is not inconsistent with our previous report showing that an idio type of alloreactive T-cells is controlled by the Igh allotype as well as by H-2 (ref. 6). One critical difference is that, whereas in the present work an antiserum to immunoglobulin V_H determinants was used, the previous report used an antiserum against T-cell receptors which presumably detects unique determinants on T-cell receptors in addition to those shared with immunoglobulin H chains. The

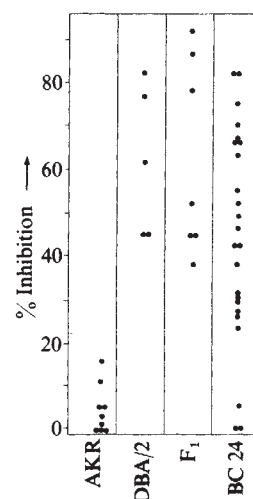


Fig. 1 Inhibition of the TNP-PFC responses of 10^6 spleen cells from individual mice immunized 3–6 weeks previously with 4×10^9 Strep.A particles, cultured for 4 days in 100 μ l Click's medium in microwells together with TNP-Strep.A¹². Sixteen identical microcultures were run of each mouse, eight of which contained 20 μ g anti- V_H antibody. Each point represents the result of a single mouse and gives the inhibition of the mean PFC response of the cultures containing anti- V_H as a percentage of that of cultures without anti- V_H . Absolute PFC responses were of the order of those shown in Tables 2 and 3.

Table 3 Allotype linked segregation of helper cell inhibition by anti- V_H using partially helper cell-depleted spleen cells of (AKR $\times$ DBA/2) F_1 $\times$ AKR backcross mice

BC	Sex	Coat colour	Thy-1*	Lyt 1*	H-2*	Immunoglobulin Igh*	TNP-PFC per 10^6 cells†		% Inhibition
							-a V_H	+a V_H	
1	♀	C/c	2/1	1/1	k/k	d/c	250(1.15)	102(1.07)	59
2	♀	C/c	2/1	2/1	k/k	d/c	344(1.31)	168(1.04)	51
3	♀	C/c	2/1	1/1	k/k	d/c	109(1.24)	46(1.24)	57
4	♀	C/c	2/1	2/1	k/k	d/d	215(1.09)	189(1.04)	12
5	♀	C/c	2/1	1/1	k/d	d/d	322(1.23)	351(1.15)	-9
6	♀	c/c	2/1	1/1	k/k	d/d	150(1.16)	140(1.24)	6
7	♀	c/c	2/1	2/1	k/d	d/d	275(1.10)	299(1.16)	-9
8	♀	c/c	1/1	1/1	k/k	d/c	320(1.21)	150(1.15)	53
9	♀	c/c	1/1	2/1	k/d	d/d	220(1.10)	202(1.10)	8
10	♀	c/c	2/1	1/1	k/d	d/d	146(1.13)	142(1.16)	3
11	♀	c/c	1/1	2/1	k/k	d/c	316(1.36)	126(1.10)	60
12	♀	c/c	2/1	1/1	k/k	d/d	93(1.30)	93(1.32)	0
13	♀	c/c	2/1	1/1	k/d	d/d	408(1.21)	412(1.12)	-1
14	♀	c/c	1/1	2/1	k/d	d/d	261(1.13)	215(1.11)	17
15	♂	c/c	2/1	1/1	k/d	d/d	334(1.27)	264(1.05)	-8
16	♂	c/c	1/1	1/1	k/d	d/d	193(1.04)	183(1.08)	5
17	♂	c/c	1/1	1/1	k/k	d/d	222(1.07)	184(1.16)	17
18	♂	c/c	2/1	2/1	k/k	d/d	195(1.08)	169(1.13)	13
19	♂	c/c	2/1	2/1	k/d	d/c	99(1.15)	36(1.35)	63
20	♂	C/c	1/1	1/1	k/k	d/d	460(1.10)	389(1.09)	15
Het/hom	6/14	6/14	13/7	8/12	9/11	6/14			

* Determined as described in Table 1 legend.

† See Fig. 2 legend.

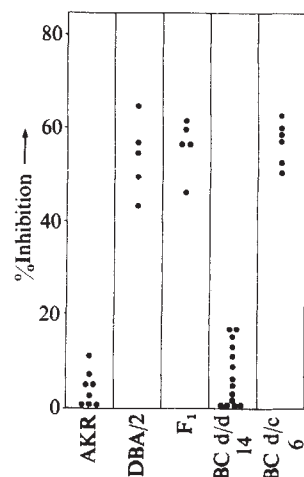


Fig. 2 Graphic representation of the data from Tables 2 and 3. The experiment was done as represented in Fig. 1, with the following modifications: cultures contained 3×10^6 cells in 300 μ l medium 20% of which were spleen cells, 80% of which were previously treated with a Thy-1.1 and rabbit complement. Eight identical cultures of each mouse were run, four of which contained 5 μ g anti- V_H antibody.

other difference that may be critical is that in our previous report the T-cell receptors investigated were specific for major histocompatibility complex antigens⁶.

It has come to our attention that other helper cell systems are not inhibited by anti- V_H (A. Coutinho, personal communication). We conclude that anti- V_H may react with only a

proportion of T-helper cell receptors, either by masking of the V_H determinant on some T-cells by combination of V_H with other structural elements in analogy to its masking by light chains on antibodies¹³, or by preference of anti- V_H for what may reflect V_H subgroups on T cells. The partial inhibition and the pronounced individual variability seen in many of our experiments¹² may be explained by subgroup preferences of the anti- V_H antibody and variability in the proportions of such V_H subgroups activated in different individuals and in response to different antigens. Taken together, we feel that antibodies to V_H framework determinants may become important in the analysis of T-cell receptors, particularly as far as their biochemical analysis is concerned.

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Murine leukaemogenesis: monoclonal antibodies to T-cell determinants arrest T-lymphoma cell proliferation

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We have proposed a receptor-mediated leukaemogenesis hypothesis wherein T lymphomas would be clones of T cells bearing mitogen-linked surface receptors specific for the envelope determinants of the inducing MuLV¹⁻³. A prediction of the hypothesis is that T-lymphoma proliferation is dependent on continued presentation of MuLV envelope determinants to these cell-surface receptors, and that substances which interfere with receptor-virus interactions should inhibit T-lymphoma proliferation. Rat monoclonal antibodies were raised to the AKR mouse T lymphoma KKT-2, and these antibodies were screened independently for blockade of virus-binding and for cytostatic activity on KKT-2 cells. We report here that those monoclonal antibodies which block virus binding inhibit growth of KKT-2 cells *in vitro*, whereas monoclonal antibodies which bind to these cells but do not block virus binding are not cytostatic. Three of the four cytostatic antibodies detect determinants on the Thy-1 molecule, while none of the other (non-cytostatic) antibodies detect Thy-1. Antibody inhibition of KKT-2 cell growth is precluded by saturation of KKT-2 virus receptors with the inducing leukaemia virus.

Spleen cells from Fisher rats immunized with KKT-2 lymphoma cells were fused with the hypoxanthine, aminopterin and thymidine (HAT) sensitive myeloma NS-1 (ref. 4), and antibody-forming cell hybrids were grown and cloned by limiting dilution. The antibodies used in this study and their characteristics are shown in Table 1. A complete characterization of these monoclonal antibodies, the molecules recognized, and

functions on normal cells will be described elsewhere (E. Pillemer *et al.*, in preparation).

To test the possible growth inhibitory function of these various monoclonal antibodies, we added them to logarithmically growing KKT-2 cells *in vitro*. As demonstrated in Fig. 1, exposure to dilutions of antibodies produced by clones 31-11, 31-8, 42-21 and 19XE5 resulted in greatly diminished thymidine uptake, whereas antibodies from clones 43-17, 43-13,

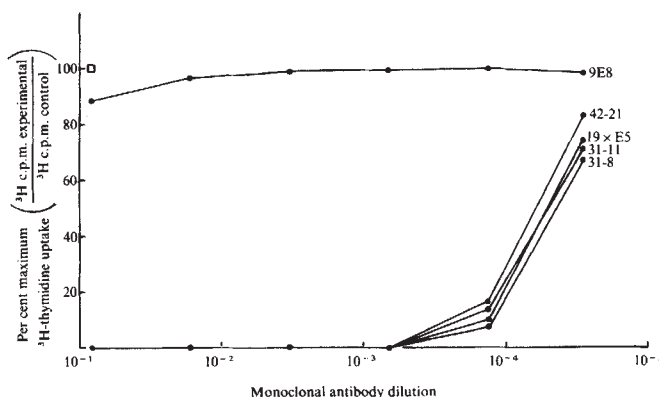


Fig. 1 Lymphoma cell growth inhibition assay. KKT-2 lymphoma cells were pelleted from subconfluent cultures and resuspended in serum-free growth medium (MEM, GIBCO) for 2 h at 37°C at a density of 10^5 cells ml^{-1} . After repelleting, the cells were resuspended in cold tissue culture medium (MEM, 10% FCS) at a density of 2×10^4 cells ml^{-1} . 5×10^4 Cells (2.5 ml) were placed in the bottom of 25-cm² Corning tissue culture flasks and serial dilutions of monoclonal antibodies were added. After growing for 14 h at 37°C, 0.1 ml of medium containing 10 μ Ci ³H-thymidine (NEN) was added to each culture for 2 h. Labelled cells were washed, 5% TCA precipitated, and per cent growth inhibition was calculated using cells without antibody as equal to 100% growth. KKT-2 cells were tested for growth inhibition with: a, anti-Thy 1 (19XE5, 30-H12, 31-11, 42-21); b, anti-MuLV (16B7, 9E8); and c, anti-cell surface antibodies (31-8, 43-17, 43-13). Data represent averages of three experiments. Similar data result when 19XE5 and 16B7 antibodies purified from hybridoma supernatants (by protein A columns)¹⁵ were utilized. □, Represents 1:12 dilutions of non-inhibitory antibodies, 43-13, 43-17, 16B7 and 30-H12.

Table 1 Analysis of monoclonal antibody specificity

Antibody	Isotype	Cell-binding RIA						Virus RIA			Ref.
		KKT-2	L691	L691/K	AKR/J NTHY	CXB/J NTHY	KKT-2 SL	MCF 247	Molecular weight	Proposed specificity	
31-8	IgG _{2A}	+++	—	—	+++	+++	—	—	80,000	T80	5
31-11	IgG _{2A}	+++	+++	+++	+++	+++	+++	—	27,000	Thy-1 constant	5
43-13	IgG _{2B}	++	—	++	—	—	+	—	95,000	Cell surface	
									85,000		
43-17	IgG _{2B}	++	—	++	—	—	—	—	50,000		
42-21	IgM	+	+	+	+	+	—	—	27,000	Thy-1 constant	
30-H12	IgG _{2B}	—	+++	+++	—	+++	—	—	27,000	Thy-1.2	14
9E8	IgG _{2A}	++	—	++	—	—	+/-	—	15,000	p15E	15
19XE5	IgG ₁	+++	—	—	+++	—	++	—	27,000	Thy-1.1	5
16XB7	IgG _{2A}	+/-	—	—	—	—	—	—	70,000	eco gp70	15

Monoclonal antibodies (31-8, 31-11, 43-13, 43-17 and 42-21) were prepared by a modification of the technique of Galfré *et al.*⁴. Briefly, Fisher rats were immunized with KKT-2 lymphoma cells; three days following the final intravenous injection, the spleen (approximately 2×10^8 cells) was fused with 10^7 NS-1 myeloma cells using 38% polyethylene glycol-1540. Hybrids were grown initially in super HAT medium¹² in 24-well plates (Falcon 3008). Supernatants were tested for binding to a panel of tumour cells, normal lymphoid cells and purified virus using a conventional radioimmunoassay (RIA) with a ¹²⁵I-labelled rabbit anti-rat second stage as the detecting reagent. MCF-247 and KKT-2-SL viruses were prepared by Sepharose 4B chromatography¹³. Molecular weight determinations were performed as previously described⁵. Specificities for most of the antibodies have been described previously; work is continuing to characterize molecules recognized by the 43-13, 43-17 and 42-21 monoclonals. Clone 31-8 has been lost. Identity of tumour cell lines: (1) KKT-2 is a spontaneous AKR/J lymphoma; (3) L691/K is the L691 lymphoma super-infected with the KKT-2-SL virus⁹. +, Relative level of binding as determined by RIA; —, indicates that binding was at the level of background obtained when using the second stage reagent alone. Rat monoclonal 30-H12 was provided by J. Ledbetter¹⁴. Mouse monoclonals 9E8, 19XE5 and 16B7 were provided by R. Nowinski¹⁵.

16B7, 9E8 and 30-H12 were without effect on DNA synthesis. Growth inhibitory antibodies block DNA synthesis significantly at concentrations to 1:10,000, while cell binding, non-inhibitory antibodies are ineffective, even at concentrations approaching 1:10. This inhibition was not related to antigen mobility as the inhibitory antibodies show different patching and capping activity in the presence of second stage antibodies; 19XE5 and 42-21 caused rapid membrane capping, 31-8 intermediate capping activity, and 31-11 caps very slowly and incompletely (data not shown). The inhibition was extremely cell density dependent, and was optimal with exponentially dividing cells at less than 2×10^4 cells per ml. At 2×10^5 cells per ml, even 1:10 dilutions of 31-11 were not inhibitory.

Figure 2 shows that KKT-2 cell growth inhibition is directly related to the level of cell-surface antibody bound and degree of interference with murine leukaemia virus (MuLV) binding. Growth inhibitory levels of 31-11 and 42-21 (>50% saturation) significantly blocked MuLV binding whereas the non-inhibitory 43-17 antibody did not.

The effect of growth inhibitory antibodies seems to be most active in cell-cycle phase G₁. We have previously shown that after exposure to 31-11 antibody, KKT-2 cells show a linear fall to background in their ability to synthesize DNA within 8 h with a shift in DNA content from 4n towards 2n copies within 20 h. This corresponds to a shift in size from large lymphoblastic to small G₀-type lymphoid cells within 24 h, culminating in non-complement mediated cell death within 48 h⁵.

If growth inhibition by particular monoclonal antibodies is mediated by steric or direct blockade of receptor-viral envelope interactions, then preincubation of lymphoma cells with their inducing retrovirus ought to fill such receptors, preventing antibody-induced growth inhibition. To test this possibility, we preincubated KKT-2 lymphoma cells with saturating concentrations of their own endogenously produced SL retroviruses, or with various viruses closely or more distantly related (as defined by host of origin⁶, shared and distinct antigenic determinants⁷, and cell-binding specificity^{3,8}). We then added monoclonal antibodies to these mixtures at growth inhibiting concentrations. The results, shown in Table 2A, were striking: only the endogenously produced retrovirus (KKT-2-SL) prevented antibody-induced growth inhibition of KKT-2 cells. Table 2B demonstrates that this inhibition is not secondary to a direct interaction of antibody and virus, as preadsorption of limiting concentrations of antibody with a great excess of virus does not prevent growth inhibition. We have demonstrated elsewhere that pre-binding of KKT-2 cells with saturating levels of virus does not alter the amount of 31-8 or 31-11 antibodies bound to these cells⁵.

To test whether live viruses are necessary for continued cell growth in the presence of inhibitory antibodies, we preirradiated

KKT-2-SL virus with doses of UV known to inactivate >99.99% viral replicating and infectious activity⁹ before pre-binding. This radiation dose did not affect the ability of these virions to prevent growth inhibition by monoclonal antibody (Table 2C).

These experiments demonstrate that T-lymphoma cells, like T lymphocytes, are under cell-surface regulation for stimulation and cessation of DNA synthesis. It is likely, therefore, that leukaemogenic retroviral oncogene action involves stimulation of cell-surface mitogen receptors, alone or in combination with other intracellular signals. These latter signals may also be under viral genetic control, either by direct expression of viral genes or by selective activation of host genes. In this view cell-surface stimulation would be necessary, but not sufficient for lymphoma cell proliferation. As a corollary, lymphoma cell growth

Table 2 Inhibition of antibody-induced KKT-2 cell growth arrest

	Per cent control ³ H-thymidine uptake	
	31-11	31-8
A Preincubated virus:		
PBS	2	2
KKT-2-SL	85	96
MCF-247	4	6
L691/G	5	3
BL/Ka[X]	7	4
BL/Ka[B]	4	3
L691/M	8	7
B Antibody preabsorbed with virus:		
PBS	5	6
KKT-2-SL	11	8
L691/M	8	7
BL/Ka[B]	8	5
C UV inactivated KKT-2-SL virus		
	75	105
D Control cells without inhibitory antibody addition		
	100	100

The standard KKT-2 cell growth inhibition assay as outlined in Fig. 1 legend was carried out with 31-11 and 31-8 antibodies at a 1:250 dilution on 5×10^4 KKT-2 cells after preincubation with purified retroviruses. **A**, 0.01 A₂₆₀ unit of Sepharose-4B purified virus¹³ in 0.3 ml phosphate buffered saline (PBS) was incubated with KKT-2 cells for 60 min. at room temperature before addition of inhibitory antibodies. This amount of virus represents a receptor saturation level as previously determined⁸. The origin of each retrovirus population has also been previously described^{3,8}. **B**, One A₂₆₀ unit of Sepharose purified KKT-2-SL, L691/M and BL/Ka[B] virus in PBS was incubated with 31-11 and 31-8 antibodies at a 1:2.5 antibody dilution for 60 min at room temperature with PBS control absorbed samples. The samples were spun at 100,000g for 120 min to pellet virus, and the absorbed antibodies were tested in the standard KKT-2 cell growth inhibition assay (Fig. 1). All inhibitions were carried out at a 1:250 final antibody dilution. **C**, Sepharose-4B purified KKT-2-SL virus was UV inactivated and used to inhibit antibody induced KKT-2 cell growth inhibition as in **A**. Virus (5 ml) in PBS (1 A₂₆₀ unit ml⁻¹) was irradiated for 145 s at 4,000 erg mm⁻² before use. **D**, Control growth inhibition of KKT-2 cells in the absence of inhibitory antibodies.

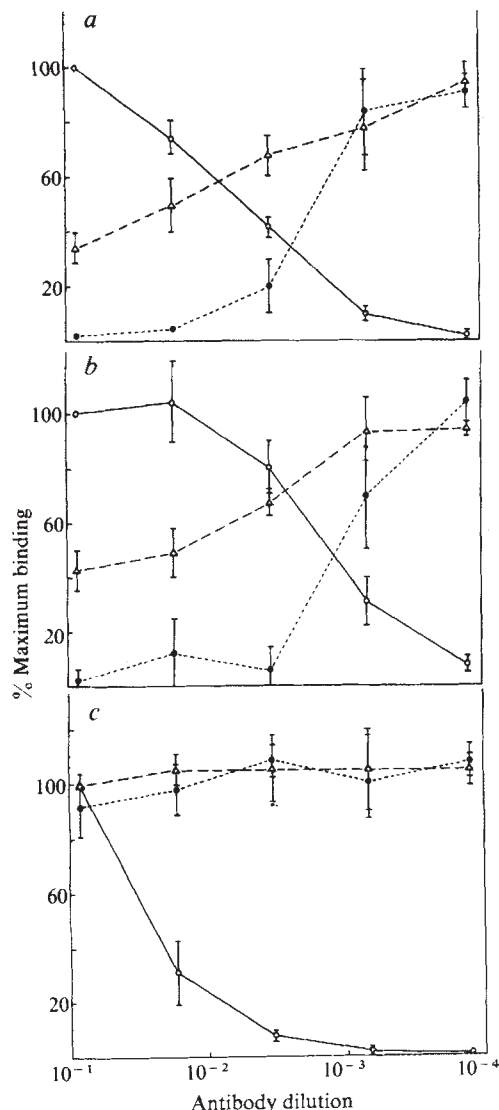


Fig. 2 Effect of serial dilutions of monoclonal antibodies prebound to KKT-2 cells on growth inhibition and MCF-247 virus binding. 10^4 KKT-2 cells were suspended at 2×10^4 cells per ml in serial dilutions of monoclonal antibody supernatants: a, 31-11; b, 42-21; c, 43-17, for 1 h at 0°C before washing through neat fetal calf serum. Fluoresceinated MCF-247 binding and detection of cell-surface antibody by fluorescence-activated cell sorter analysis was carried out on 4.75×10^5 cells each as previously described⁵. 5×10^4 cells from each point were suspended in 2.5 ml of MEM/10% FCS and growth inhibition was determined as in Fig. 1 legend. 100% binding of MCF-247 was that level bound to KKT-2 cells treated in parallel without antibody. 1:1 nonfluoresceinated MCF-247 inhibition of binding showed a 43% inhibition of fluoresceinated MCF-247 binding. 100% antibody binding was that level bound to cells at the lowest dilution (1:12). Antibody binding (○—○); MCF-247 binding (△—△); (^3H -thymidine uptake experimental/ ^3H -thymidine uptake control) $\times 100\%$ (●—●). The curves represent data from three experiments $\pm$ standard deviation.

inhibitory antibodies should also inhibit antigen-induced proliferation of normal T-cell subsets. In fact, we present elsewhere evidence that the anti-Thy-1 monoclonal antibodies may inhibit concanavalin A mitogen and alloantigen induced T-cell proliferation (N. Hollander *et al.*, in preparation).

These experiments provide strong evidence that cell-surface MuLV-specific receptors on T lymphomas are involved in continued proliferation by these cells, as predicted by the receptor-mediated leukaemogenesis hypothesis^{1,3}. The hypothesis, however, is not proven: for example, growth-inhibiting antibodies may affect cell proliferation by binding to molecules which generate antimitotic signals independent of viral receptors. Thus, prebound virus might inhibit antibody binding to a subset of antimitotic molecules, providing that the antimitotic subset is located near viral receptors. It is also known that some antigen-activated T cells may respond to T cell growth

factors by continued proliferation (in the presence or absence of antigen¹⁶). It is not yet known if KKT-2 cells belong to that category of cells, but the relative resistance of cells grown at higher density to growth inhibition by these antibodies might be due to these factors, or to higher concentrations of viral envelope determinants. Another potential difficulty with the hypothesis is the incidence of aneuploidy (trisomy 15) in AKR and RadLV-induced leukaemias¹⁰. While it is conceivable that this is not related to the leukaemogenic process, it seems more likely, that it is a necessary event in the progression to leukaemia¹¹. Whether this is a necessary event related to cell-surface stimulation or some other aspect of unlimited cellular proliferation is still unclear.

It is interesting to speculate that other neoplastic cell types might also be regulated by stimulation of cell-surface mitogen-linked receptors by self-produced (or exogenous) complementary ligands. These cells also might be susceptible to growth inhibition by appropriate monoclonal antibodies.

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Lateral diffusion of lipopolysaccharide in the outer membrane of *Salmonella typhimurium*

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Gram-negative enteric bacteria are enveloped by two membrane systems¹. The inner or cytoplasmic membrane is responsible for the major metabolic functions including biosynthetic activities², while the major known functions of the outer membrane are primarily physical: it contains receptors for bacteriophages and bacteriocins³; it contributes to the maintenance of cell shape⁴; and it controls access of nutrient solutes and agents such as antibiotics and detergents to the cytoplasmic membrane⁵. Several investigations have indicated that mobility of membrane components, particularly lipopolysaccharide^{6,7}, is essential for biogenesis of the outer membrane^{7,8}, and is a primary

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event in phage infection^{9,10}. To define more accurately the fluid dynamic properties of the outer membrane as related to function, we have now developed the capability to measure lateral diffusion coefficients *in vivo* of rhodaminated G30 lipopolysaccharide fused into *Salmonella typhimurium* G30A filamentous bacteria. The method used extends the FRAP procedure (fluorescence redistribution after photobleaching)^{11,12} to bacteria and the results demonstrate rapid diffusion of lipopolysaccharide ($D = 2.0 \pm 0.9 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$) over micrometre distances.

In the FRAP technique, lateral transport parameters are determined from the characteristic redistribution of fluorescent label after a gradient of concentration is established by a localised photobleaching pulse. Gram-negative bacteria are in general too small to be studied successfully by this approach, but mobility measurements can be performed on bacterial filaments. Nonseptate filaments 50–100 μm long, consisting of a syncytium of bacteria sharing a common, continuous inner and outer membrane, were obtained by treatment with cephalixin¹³ (see Fig. 1).

Exogenous fluorescently labelled lipopolysaccharide was incorporated into the bacterial outer membrane by vesicle fusion, following methods devised by Jones and Osborn^{14,15}. The lipopolysaccharide was isolated from *S. typhimurium* G30 by the method of Galanos¹⁶, further purified by electrodialysis¹⁷, and reacted with rhodamine isothiocyanate following the procedure of Schindler *et al.*¹¹. Unilamellar phospholipid vesicles containing the lipopolysaccharide were prepared, and incubated with a preparation of bacterial filaments for 40 min, at 37 °C. Jones and Osborn^{14,15} have demonstrated that this procedure transfers lipopolysaccharide to the outer membrane of the acceptor bacteria. While vesicle derived phospholipid translocates to the inner membrane, the lipopolysaccharide does not, and resides exclusively in the outer membrane^{14,15}.

Electron microscopy was used to examine the surface distribution of vesicle derived lipopolysaccharide (see Fig. 2). For this purpose, lipopolysaccharide was derivatised with 2,4,6-trinitrobenzene sulphonic acid (TNBS) in order to incorporate trinitrophenol hapten (TNP) groups into the molecule. Vesicles

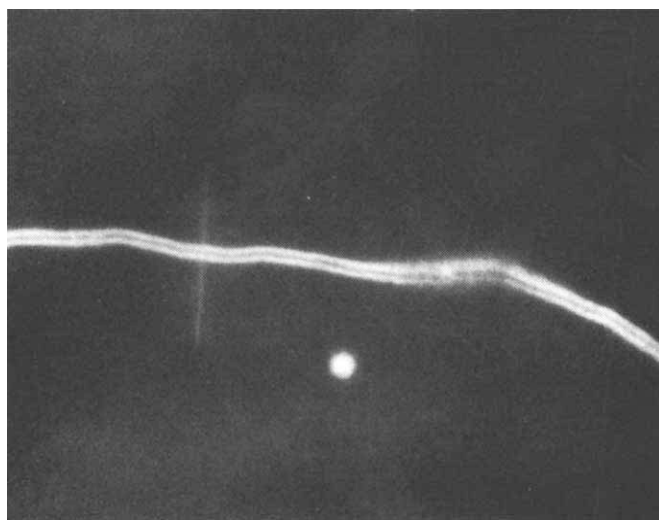


Fig. 1 Bacterial filament viewed under dark-field light microscopy. *S. typhimurium* G30A was grown in PPBE from an overnight culture at a dilution of 1/20. After one generation of normal growth cephalixin was added to the medium ($10 \mu\text{g ml}^{-1}$). The culture was shaken vigorously and allowed to grow to $A_{600} = 1.0$. The culture was then diluted 1/10 in PPBE containing cephalixin ($10 \mu\text{g ml}^{-1}$) and again allowed to grow to $A_{600} = 1.0$. The cells were collected and washed with a 10 mM HEPES/cephalexin buffer pH 7.5. Superimposed on the filament, by photographic double exposure, is a 'picture' of the incident focused laser beam produced by imaging the fluorescence excited in a thin layer of concentrated dye solution.

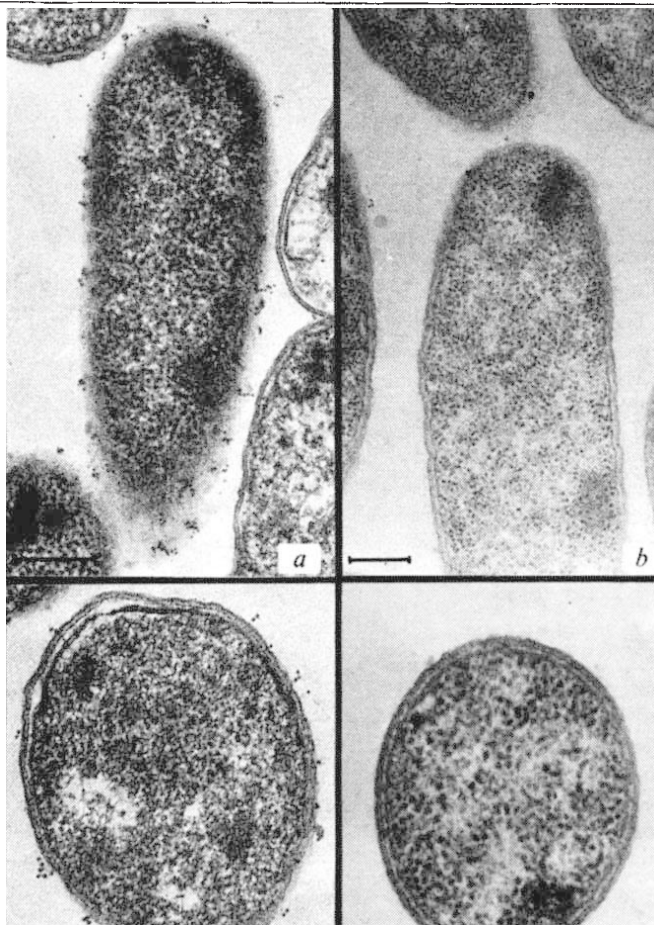


Fig. 2 Electron microscopy of *S. typhimurium* with and without exogenously added TNP-lipopolysaccharide. Freshly prepared TNP-lipopolysaccharide-phospholipid vesicles (phospholipid/lipopolysaccharide ratio = 60:1) were prepared and incubated with cells as described elsewhere^{13,14}. TNP-lipopolysaccharide G30 was prepared as follows: 5 mg G30 lipopolysaccharide was reacted with 5 mg trinitrobenzene sulphonic acid (TNBS) in 1 ml 0.2 M NaHCO_3 , pH 8.5 at room temperature for 1 h; the reaction mixture was applied to a Sephadex G-25 column ($1.5 \times 20 \text{ cm}$) and eluted with water; the void volume peak was lyophilised and stored at 4 °C. Goat anti-DNP antibodies ($20 \mu\text{l}$, 1.5 mg ml^{-1}) were added to the bacteria suspended in 1 ml, 50 mM HEPES pH 7.5 and allowed to incubate at room temperature for 5 min. The cells were washed three times with 2 ml buffer and resuspended in 1 ml of the same buffer. Ferritin-labelled rabbit anti-goat IgG antibody (0.2 ml, Cappel Labs) was added to the cells for 5 min at room temperature. The cells were then washed again three times in buffer, pelleted and fixed in 2% glutaraldehyde at 4 °C for 1.5 h. Thin section preparation was performed as described previously¹⁴. *a* and *b* are longitudinal sections while *c* and *d* are transverse sections of *S. typhimurium* with (*a*, *c*) and without (*b*, *d*) TNP-lipopolysaccharide. Scale bars, 220 nm.

were prepared and fused with non-filamentous *S. typhimurium* in the usual manner. After fusion, goat anti-DNP antibodies were reacted with the bacteria followed by incubation with ferritin-labelled rabbit anti-goat IgG antibodies. The samples were fixed in glutaraldehyde and thin sectioned for examination. Figure 2 demonstrates that the vesicle derived exogenous lipopolysaccharide is incorporated in the bacterial outer membrane.

Diffusion coefficients were determined by the FRAP technique using a recently introduced 'multi-point' analysis¹². The labelled lipopolysaccharide was photobleached with a laser beam focused to a narrow band ($\sim 1 \mu\text{m}$ wide) aligned to intercept one or more bacterial filaments (see Fig. 1). The redistribution of mobile molecules along the filaments was then followed by monitoring the fluorescence intensity excited by an attenuated beam ($\times 5 \times 10^{-5}$) sequentially positioned at each of several locations in and adjacent to the bleached area.

Recovery curves of rhodamine-labelled G30 lipopolysaccharide in the acceptor bacteria G30A are shown in Fig. 3. From the functional form of the fluorescence recovery, it is clear that all the exogenously added lipopolysaccharide is free to diffuse in the outer membrane with no immobile components (recovery is greater than 90%). From a nonlinear least squares fit of the data to the appropriate theoretical equations¹², a value of $2.0 \pm 0.9 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ was found for exogenously added lipopolysaccharide. Following addition of anti-G30 antibody, recovery is still monophasic, but now is very much slower with a calculated diffusion constant of $2.0 \pm 0.4 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$. This clearly indicates that the majority of lipopolysaccharide is situated in the outer membrane accessible to antibody molecules.

Mühlradt *et al.*⁶, using indirect techniques, suggested that lipopolysaccharide diffusion is much slower ($D \leq 3 \times 10^{-13} \text{ cm}^2 \text{ s}^{-1}$). A direct comparison of our results is not possible because those experiments were performed with wild-type bacteria in which the carbohydrate side chain on the lipopolysaccharide is much longer¹. In addition, wild-type bacteria have approximately 2.5 times more protein in the outer membrane¹⁸. The effect of these parameters on lipopolysaccharide mobility is now being investigated.

The relatively rapid rate of diffusion that we observe for lipopolysaccharide is in marked contrast to the immobility of matrix-porin as determined by Smit and Nikaido¹⁹. Very similar diffusion characteristics for lipopolysaccharide and matrix-porin were observed by us *in vitro*, in reconstituted outer membrane multibilayers¹¹.

This work further extends to the FRAP technique which has previously been used exclusively on eukaryotic cells. The use of this technique should provide new and fundamental information

on the molecular dynamics of the outer membrane and the relationship of these dynamics to biogenesis and function of the membrane. The bacterial system is readily manipulable by genetic and physiological means, and can be used as a model for exploration of causal relationships between the molecular composition and organisation of a membrane, its fluid dynamics and specific functions (including receptor function and signal transmission).

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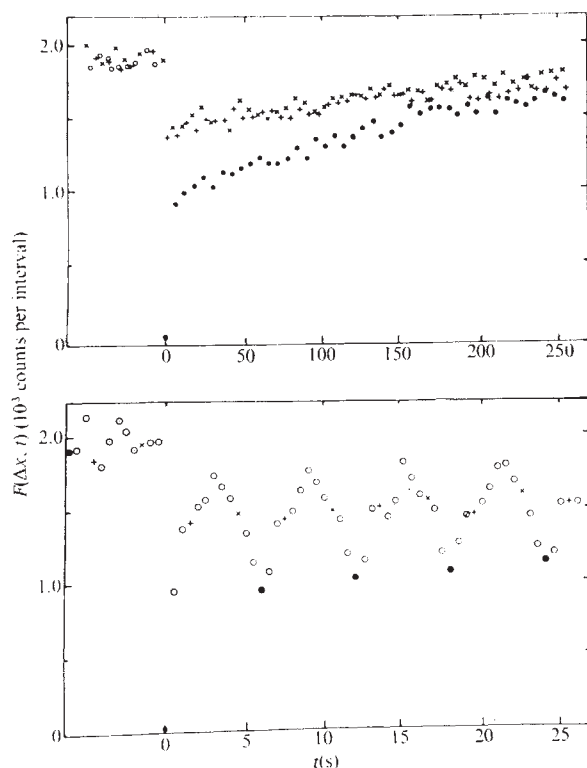


Fig. 3 Recovery curves of rhodaminated G30 lipopolysaccharide. Sample was bleached with an intense laser pulse focused to a narrow band intersecting the filament. Fluorescence intensity, $F(\Delta x, t)$, was measured as a function of position and time with an attenuated beam sequentially positioned at each of 12 equally spaced locations ($0.36 \mu\text{m}$ separation) along the filament. Top: Recoveries measured at 3 of the 12 locations: coincident with the bleaching pulse ($\circ$), and $1.08 \mu\text{m}$ on either side of centre ($+$, $\times$). Bottom: Sequential scans including all 12 locations on an expanded time scale. Data were fitted to the form of equation (9) of ref. 12, assuming a 100% recovery.

A nucleosome-free region in SV40 minichromosomes

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Simian virus 40 (SV40) DNA is found in infected cells in the form of a minichromosome¹. It possesses a beaded structure composed of cellular histones and supercoiled viral DNA in a molecular complex which is very similar to that of cellular chromatin²⁻⁴. The similarity between these structures, and the fact that the major viral functions take place in cell nuclei via cellular machinery, have made SV40 an attractive model system in which to study the organization and expression of the more complex eukaryotic chromatin. Early studies on SV40 chromatin, attempting to determine the precise distribution of the histone beads (nucleosomes) along the DNA, indicated that the nucleosomes were randomly distributed relative to the viral DNA sequences⁵⁻⁸. This picture has recently been altered by a study indicating that a region close to the viral origin of replication is particularly sensitive to nuclease digestion⁹⁻¹³. Using a variety of nucleases¹⁰⁻¹³, the nuclease-sensitive region has been shown to map on a stretch of the genome beginning at the viral origin of replication and continuing about 400 base pairs into the 'late' side of the DNA. The simplest interpretations of the presence of a nuclease-sensitive region are that this region is either deficient in nucleosomal protein or that it contains a peculiar arrangement of these protein structures. We have analysed SV40 minichromosomes in the electron microscope in an attempt to determine whether alterations in the gross nucleoprotein structure of this region could be visualized. We report here that such an alteration does exist and that about 25% of SV40 minichromosomes observed contained a region of DNA between 0.67 and 0.75 map units which is not organized into the typical nucleosome beaded structure.

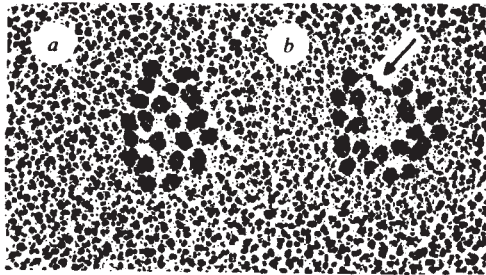


Fig. 1 Electron microscopic visualization of SV40 minichromosomes. At 48 h post-infection, SV40-infected BSC-1 cells were washed twice with 0.05 M Tris-HCl, pH 7.9, 0.001 M MgCl₂ and 0.005 M 2-mercaptoethanol (buffer H), and collected by low speed centrifugation. The cell pellet was resuspended in buffer H and centrifuged again at low speed. This procedure caused lysis of about 95% of the cells. The nuclear pellet was resuspended in 1 ml buffer H per 10⁷ nuclei and incubated at 37 °C for 30 min with occasional shaking. The suspension was then centrifuged at 10,000 g for 1 min and the supernatant containing the SV40 chromatin was run through a 5–30% (w/w) sucrose gradient in buffer H containing 0.1 M NaCl for 2 h at 32,000 r.p.m. in the Spinco SW41 rotor at 4 °C. The 75S peak³⁰ (minichromosomes) was pooled, and immediately mounted on carbon membrane-coated grids activated by glow-discharge³¹. The grids were stained with 2% uranyl acetate and rotary shadowed with Pt/Pd (80:20). Molecules were visualized with a Philips 300 electron microscope, and photographs were taken at a magnification of $\times 42,000$. *a*, Minichromosome without a gap; *b*, minichromosome with a gap, indicated by the arrow.

Figure 1 shows the appearance of representative SV40 minichromosomes with and without an exposed region ('gap') of DNA. Reproducibly we found that approximately 25% of the readable molecules contained a single gap, whereas molecules containing more than one gap comprised less than 1% of the population. We have measured the length of the 'exposed' region relative to naked SV40 DNA spread in the same sample. Figure 2 shows that the average length was $103 \pm 30 \mu\text{m}$. Assuming the DNA to be fully extended, this gap corresponded to 320 ± 90 base pairs of DNA. However, this length is a minimum estimate, as the spreading method used could not detect on the exposed DNA proteins that were able to cause the DNA's condensation.

To locate the gap relative to restriction sites on the physical map of SV40 DNA, minichromosomes were digested with site-specific, single-cut, restriction endonucleases. The extent of minichromosome linearization was dependent on the particular enzyme used. Digestion with either *Bam*HI or *Eco*RI converted only 20–30% of the minichromosomes to linear molecules, even in conditions of enzyme excess. Digestion with *Bgl*II produced 60–70% linear molecules. Similar proportions of linear molecules were observed when DNA extracted from minichromosomes digested by these restriction endonucleases was analysed by electrophoresis on agarose gels.

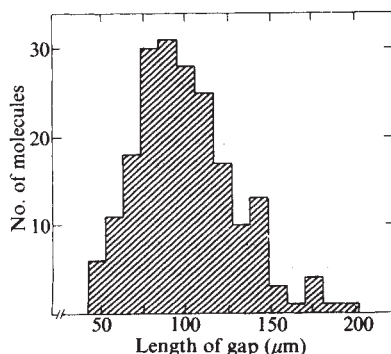


Fig. 2 Histogram of the length of the gap in the minichromosome. The length of the gap was measured in molecules as shown in Fig. 1*b*. SV40 DNA was used as internal length standard ($1.7 \mu\text{m}$).

Figure 3 shows the appearance of representative linear molecules that contain gaps, and Fig. 4 is a histogram showing the distribution of nucleosomes about the gap. When minichromosomes were cleaved with *Bam*HI, the exposed DNA stretch was located between short and long beaded arms, containing an average of 9 ± 1 beads and 11 ± 1 beads, respectively (Figs 3*a*, 4*a*). Similarly, when minichromosomes were treated with *Eco*RI, the gap resided between a short arm with an average of 6 ± 1 beads and a long arm with an average of 14 ± 1 beads (Figs 3*b*, 4*b*). Cleavage with *Bgl*II yielded a stretch of exposed DNA on the end of a string of nucleosomes averaging 19 ± 1 beads (Figs 3*c*, 4*c*). Occasionally, we observed a solitary bead on the far end of the open stretch after treatment with *Bgl*II (see arrow, Fig. 3*c*). These results unequivocally locate the exposed stretch of DNA as starting at or very close to the *Bgl*II restriction site and extending into the late region for at least 320 base pairs. The high rate of cleavage by *Bgl*II indicated that in most molecules, the *Bgl*II cleavage site was not blocked by a nucleosome.

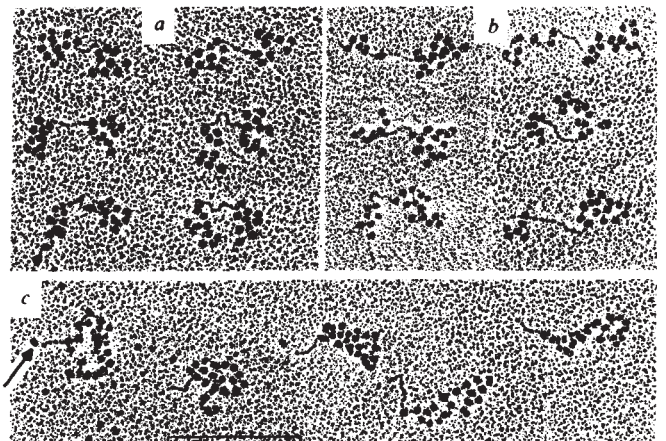


Fig. 3 Electron microscope visualization of SV40 minichromosomes cleaved with restriction endonucleases. The minichromosomes present in the 75S peak³⁰ of the sucrose gradient (see Fig. 1 legend) were diluted into 1 volume of a solution containing 0.02 M MgCl₂ and 0.002 M dithiothreitol, and digested for 60 min at 37 °C with *Bam*HI, (*a*), *Eco*RI (*b*) and *Bgl*II (*c*), before mounting for electron microscope visualization as in Fig. 1. The cleavage sites are: *Bam*HI, $0.15 \mu\text{m}$; *Eco*RI, $0.0 \mu\text{m}$ and *Bgl*II, $0.67 \mu\text{m}$ (see ref. 17). *Bgl*II cuts at the origin of replication¹⁸.

Molecules without a gap possessed an average of 21 ± 2 beads, regardless of whether or not they were linearized by restriction enzymes. Minichromosomes with a gap possessed an average of 20 ± 2 beads, again independent of their linear or circular structure. Thus, cutting the minichromosomes with restriction endonucleases did not cause the loss of nucleosome structure due to slipping at the ends. The number of nucleosomes observed on the SV40 minichromosomes seemed to be similar in molecules with and without a gap. However, as the distribution of the number of nucleosomes is broad, we cannot distinguish between the possibility that the gap was generated during preparation by loss of one or two nucleosomes and the possibility that this site was devoid of nucleosomes *in vivo*. In the first case, the nucleosome(s) lost might be different structurally from those that remained associated with the DNA of the minichromosomes. One possibility is that they contain modified histones, which could cause the histone octamer (nucleosome) to dissociate easily from the DNA. It is also possible that in this region of the SV40 genome there is a transient binding of nucleosomes, or parts of nucleosomes, to only one strand of the DNA, as was recently proposed for eukaryotic chromatin by Palter *et al.*¹⁴. They suggested that this mechanism requires a major conformational change in the nucleosome, and we believe that such a change, if it occurs, may render the nucleosome unrecognizable in the electron microscope¹⁵. The exact mode of histone

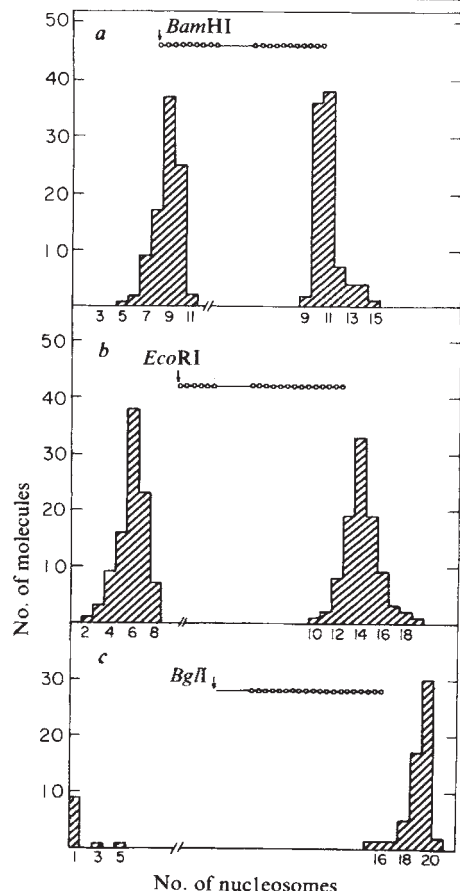


Fig. 4 Histograms of the number of nucleosomes on each side of the gap in the cleaved minichromosomes. The analysis was carried out on molecules as in Fig. 3. The inserts diagram the linearized molecules showing the nucleosomes about the exposed region relative to the enzyme cutting site.

binding to single-stranded DNA may facilitate some, or all, of the regulatory signals that have been mapped to the exposed region of the viral DNA. These include the origin of DNA replication¹⁶, the promoters for late transcription¹⁷, the heterogeneous 5' ends of the late mRNA^{18,19}, the cap structures at the 5' ends of the late mRNAs²⁰⁻²² and a major binding site for viral T antigen^{23,24}. That *Escherichia coli* RNA polymerase preferentially initiates transcription *in vitro* in this region of the SV40 minichromosome and asymmetrically transcribes the late strand (E.B.J., Y.A., S. Saragosti and M. Yaniv, unpublished results) also may support this possibility. In addition, the observation that transcriptionally active regions in eukaryotic chromatin are preferentially susceptible to both DNase I²⁵⁻²⁷ and staphylococcal nuclease digestion²⁸ also indicates that similar 'gapped' structures may exist in cellular chromatin¹⁵. It should be emphasized, however, that we have not demonstrated that SV40 minichromosomes with an exposed region are the transcriptionally active viral chromatin. Although others have concluded that only a small fraction (0.5–3%) of the SV40 minichromosomes is transcriptionally active²⁹, we have observed that a much larger fraction of the minichromosomes is transcribed (unpublished). We are now investigating the correlation between gapped minichromosomes and transcription.

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Decreased rate of DNA-chain growth in human basal cell carcinoma

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The DNA synthetic (S) phase of cell division is about twice as long in human basal cell carcinoma (BCC) as in the normal epidermis and several non-malignant skin diseases¹⁻³. A similar increase in S phase duration seems to occur in tumours of the human oesophagus⁴, larynx⁴ and recto-colon^{5,6}. At the molecular level, the overall rate of replication in a nucleus is determined by two factors—the frequency of initiation of replication and the rate of replication fork progression along the units of replication (for review see refs 7,8). Here we present evidence (based on DNA fibre autoradiography⁹⁻¹¹) that the observed increase in S phase duration may be due to a marked reduction in the rate of DNA replication along replication units, the density of simultaneously operating replication units remaining unchanged. This indicates an alteration in some biological component(s) of DNA synthesis in BCC that is of potential interest for characterizing the malignant transformation of the epidermal cell and that may perhaps be exploited therapeutically.

We used biopsy samples from five normal subjects and three patients with basal cell carcinoma, small undifferentiated tumours with a multinodular structure (nodules 50–200 cells in diameter). The tissue samples were divided into lamellae about 1 mm thick, and labelled *in vitro* with a single specific activity of ³H-thymidine. We used a previously described DNA fibre autoradiography⁹⁻¹¹ with pulse periods of 30 and 60 min (see Fig. 1 legend). This provides valid estimates of relative rates of DNA synthesis only, based on the length of grain tracks in the fibre autoradiograph^{7,12,13}. Similarly, in these conditions, centre-to-centre distances between adjacent tracks reflect the relative density of replicating units, rather than the absolute value^{7,14}.

Figure 1 summarizes the results by showing the distribution histograms of grain track lengths and centre-to-centre distances for pooled data from each tissue type. Cumulated frequency curves drawn for the two parameters allow estimation of their median value. The median track length thus obtained after the 30-min and the 60-min labelling period, respectively, were

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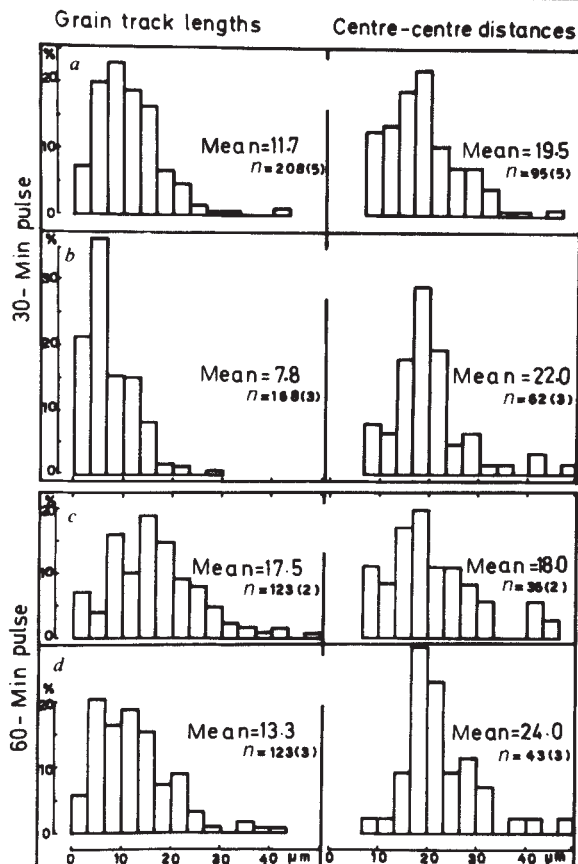


Fig. 1 Length and distance between centres of ^3H -thymidine-labelled segments in DNA molecules isolated from normal cells and from basal cell carcinoma (BCC) of the human epidermis. Biopsy samples taken from the number of patients indicated in parentheses (informed consent was received from the patients) were divided into lamellae about 1 mm thick and preincubated in Eagle's basal minimal essential-diploid medium with $2 \times 10^{-6}\text{M}$ 5-fluorodeoxyuridine (FUDR) to exhaust the endogenous thymidine nucleotide pool²⁵. The tissue fragments were then incubated with $5 \times 10^{-6}\text{M}$ ^3H -thymidine (specific activity 40 Ci mmol^{-1}), FUDR remaining in the medium. After further incubation for 30 or 60 min, the tissue samples were rinsed and incubated at 37°C for 30 min with 1% trypsin–0.3% dithioerythritol (Sigma) dissolved in phosphate buffer, pH 7.8 to separate the epidermis from the dermis²⁶. The process was furthered by a 10-s ultrasonic vibration at about 100 W using a Braunsonic 300 S apparatus (VWR Scientific). The dermis was removed by filtration through gauze, and the epithelial cells were further dissociated by another 10-s ultrasonic vibration (as above) (detachment of the dermis was assessed by microscopic examination of histological preparations prepared at this stage). DNA fibre smears were then prepared as described by Lark *et al.*²⁷. The smears (six slides per biopsy sample) were processed for autoradiography by dipping in K5-emulsion (Ilford) diluted at 50%. Exposure time was 45 days. Autoradiographs were developed for 2 min in Dektol (Kodak) and fixed. Grain track lengths and centre-to-centre distances were measured using a micrometric eyepiece and disregarding both end segments of tandem arrays on which measurements were effected. The results in each group were pooled (n = total number of measurements obtained) and the mean is indicated. With both pulse durations the difference in the mean track length between BCC and normal epidermis was highly significant ($P < 0.001$, Wilcoxon's rank test), whereas there was no significant difference between distributions of centre-to-centre distances in any group and at any pulse duration. *a, c*, Normal epidermis; *b, d*, BCC.

10 μm (range 8–12) and 17 μm (range 15.3–17.3) for the normal epidermis and 6.3 μm (range 5.2–6.7) and 12 μm (range 9.7–12) for BCC. The higher track length measured in normal tissue does not seem attributable to a higher incidence of fusion between neighbouring units because the centre-to-centre distance (1) was similar in both groups and (2) did not change significantly with labelling time. Also, few tracks were longer than twice the mean length, contrary to what would be expected if a high number of fused tracks were being registered¹². Our data thus indicate that replication rate along DNA replication units is lower by a factor 1.5–1.6 in BCC than in the basal cells of the normal human epidermis.

To determine whether this might account for most of the relative lengthening (by a factor of about 1.8–1.9) of S phase duration in the cancer cells^{1,2}, it is necessary to consider what is known about the control of DNA replication during the S phase.

DNA replication in eukaryotic cells proceeds in a temporally and spatially regulated pattern, which seems species specific and preserved from generation to generation^{7,8,11,15–18}. Coordination of this programmed DNA synthesis implies that initiation is controlled by several sets of replication units sharing the same initiator (a protein, for example¹⁸) and/or a homologous recognition site^{17,18}. It has been argued that the sequence of events that determines the duration of the S phase may correspond to the successive triggering of replication in sets (or 'banks') of replication units, by some event coupled to the synthesis of the preceding set in the sequence rather than by some external clock independent of DNA synthesis¹⁹. According to such a model, a reduction in the rate of synthesis along replication units would result in a delayed initiation in the successive sets of units. This would result in an overall increase in S phase duration corresponding to the sum of as many delays as there are interdependent replication sets in the sequence.

For example, for an average centre-to-centre distance between tracks of 20 μm (our data), each replication fork must travel 10 μm to ensure fusion of adjacent units. A reduction by a factor of 1.5 in the rate of DNA synthesis in BCC would in that case extend the average replication time of synchronously replicating units by about 25 min (assuming a rate of fork progression of $0.2\text{ }\mu\text{m min}^{-1}$ in the normal cell). If this were so, the observed 10-h increase in S phase duration in the tumour cells would imply that there is a sequence of 24 of the above considered sets of replication units (more, if the absolute rate of DNA synthesis in normal cells is slower), of which the initiation and the replication rate determine the S phase timing. The existence of sub-phases in S has been explicitly considered by several authors^{20–22}, but is difficult to prove because of the absence of markers for demarcating replication time zones²⁰.

However, other mechanisms, such as a change in the timing of DNA replication in a limited number of chromosomes or a difference in the process of chain termination^{7,8}, may also participate in the increase in S phase duration.

It is also possible that the phenomenon we have observed here is more pronounced in a particular sub-class of DNA (or of nuclei) that escaped detection. Indeed, our method probably leads to preferential scoring of the predominant class of DNA molecules present in the nucleus, and the most frequent nuclear type present in a given cell population. Again, based on higher nuclear fixation of ^3H -actinomycin D in BCC (indicating a state of lower chromatin repression that would be consistent with the view that this tumour is derived from immature pluripotent cells of the epidermis²³), we previously considered²⁴ that an increase in the euchromatin–heterochromatin ratio might explain the longer S phase duration in the tumours.

At this stage we may only speculate on the molecular basis of the observed difference in the rates of elongation of DNA chains between normal and malignant epidermal cells. However, its potential importance for our understanding of cancer and as a possible rationale for chemotherapeutic investigation should prompt further study of this phenomenon.

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Repeated folding pattern in copper–zinc superoxide dismutase

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Some globular proteins contain repeated structural patterns within the same polypeptide chain. Several enzymes^{1–6} have a pseudo-symmetric two-lobed architecture: a pair of connected but well separated domains with very similar structures are grouped round an approximate 2-fold symmetry axis close to the active centre. On a smaller scale the same motif may appear inside a single protein domain: the polypeptide chain folds into two successive topologically similar subdomains which interlock symmetrically and form a compact globule^{10–16}. In such a domain the two halves come into close contact round the dyad axis; as if the structural integrity of the domain depended on the interactions between its halves, while one separated subdomain could not exist as an independent folding unit. Many of these paired structures seem to have evolved from dimeric precursors by tandem gene duplication^{17–19}. They contain repeated amino acid sequences or precisely repeated structural elements^{3,6,13,20} in which equivalent sets of α -carbon atoms can be superimposed with root mean square deviations of the order of 1–2 Å. Here it is shown that copper–zinc superoxide dismutase^{21–23} contains two paired subdomains, and the significance of the repeated folding pattern is discussed.

The copper–zinc superoxide dismutase from bovine erythrocytes has a structural core of eight antiparallel β -sheet strands which can be considered as either a flattened barrel^{24,25} or a two-layered sandwich with four strands on each face (see Fig. 1A). The inter-strand connections are similar to those in the azurins^{26,27} and the immunoglobulin domain²⁸; the β -sheet topology diagram²⁹ has a local dyad which relates strands d, e to g, h in the front face and c to f in the back face.

The topological dyad suggested that it might be possible to superpose the corresponding parts of the barrel. The shape comparison matrix method^{10,30} was therefore used to compare every possible pair of α -carbon backbone segments which had a given fixed zone length. Segments were superposed by the rigid-body rotation and translation which minimized the sum of the mean square deviation distances.

Initial tests with zone lengths of 13, 21 and 55 residues showed a local dyad on the back face relating strands b and c to e and f , with a further short repeat in the front face which related Gln 47 on strand d to Glu 119 on strand g . Further trials with α -carbon distance plots for the superposed parts revealed an extensive but interrupted structural repeat round the active centre (Fig. 1B). Here there are two long loops which extend between strands d and e on one side and between g and h on the other. When the central strands d and g which are related by the topological dyad

Table 1 Repeated structure in Cu–Zn superoxide dismutase

Atoms fitted	Results
Superoxide dismutase Cu–Zn face (<i>d</i> -loop- <i>e</i> to <i>g</i> -loop- <i>h</i>)	
37 = 109	28 atom pairs
39–51 = 111–123	R.m.s. deviation 1.58 Å
53 = 125	Max. deviation 2.99 Å
56–61 = 135–140	Rotation angle 177.9°
80–86 = 142–148	Shift along axis 0.32 Å
	Axis (0.984, –0.053, 0.173)
Superoxide dismutase back face (<i>b</i> - <i>c</i> to <i>e</i> - <i>f</i>)	
14–22 = 81–89	18 atom pairs
27–35 = 91–99	R.m.s. deviation 2.07 Å
	Max. deviation 3.31 Å
	Rotation angle 172.9°
	Shift along axis 0.71 Å
	Axis (0.810, –0.043, 0.584)
Both faces (<i>c</i> , <i>d</i> -loop- <i>e</i>) to (<i>f</i> , <i>g</i> -loop- <i>h</i>)	
27–35 = 91–99	37 atom pairs
37 = 109	R.m.s. deviation 3.17 Å
39–51 = 111–123	Max. deviation 5.07 Å
53 = 125	Rotation angle 177.3°
56–61 = 135–140	Shift along axis 0.28 Å
80–86 = 142–148	Axis (0.964, –0.019, 0.264)

The angles between the first two rotation axes and the third are 5.7° and 20.5°; between the first and the second, 25.8°. The Cu²⁺ ion lies within 2.1 Å of the screw axis through the Cu–Zn face. Note that although strand *e* is matched to strand *h* in the first fit and to strand *b* in the second, it cannot reasonably have an evolutionary relationship to both. The third fit suggests that *e* is related to *h* rather than to *b*. Another way of fitting both faces is to split strand *e* so that the first half matches half of *b* and the second matches half of *h*. The r.m.s. deviation is then considerably higher at 3.72 Å.

were superimposed large parts of the surrounding loops and strands *e* and *h* also fitted one another (Fig. 2 and Table 1) so that the front face has a high degree of spatial symmetry. In these superposition experiments no account had been taken of the active site metal ions, but the copper ion was now found to lie within 2.1 Å of the dyad axis and two of its histidine ligands, His 46 and His 118, lay in symmetrically equivalent positions. The zinc ion and its ligands lie to one side of the front face, enveloped by an irregular outlying loop (residues 62–79) which has no

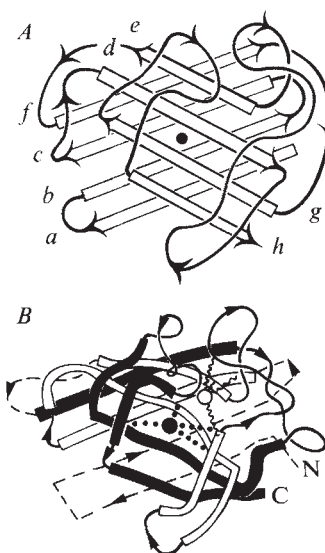


Fig. 1 β -Sheet topology. A, Connections between eight strands *a*–*h* showing front active centre face *d*, *e*, *g*, *h* with copper ion in front centre and strands *a*, *b*, *c*, *f* of back face. B, Strands *c*–*g* and loops round active centre to show the symmetry. Black and white strands are related by local dyad centred about copper ion. The open circle off-centre represents a zinc ion.

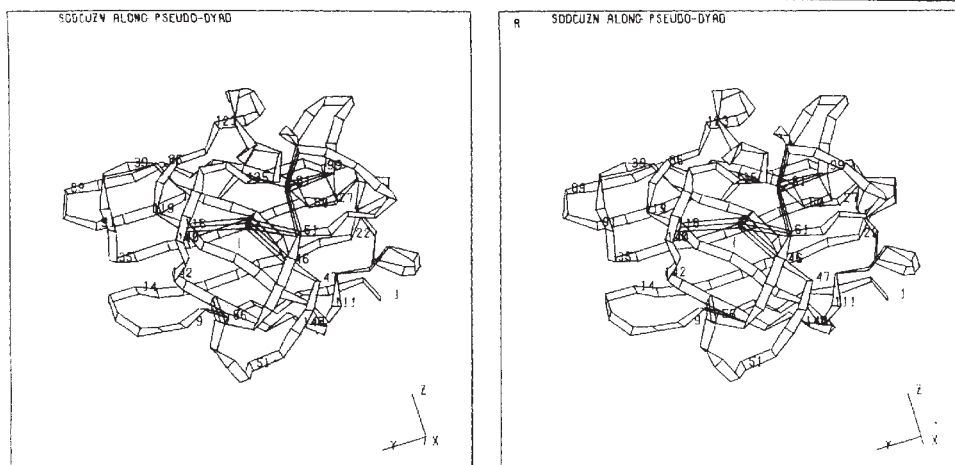


Fig. 2 Active centre face of superoxide dismutase viewed along pseudo-dyad axis (central bar). Copper ion near dyad and zinc ion to upper right are joined to ligands by triple lines. Triple line to lower left is disulphide bridge Cys 55-Cys 142. Structure repeated across pseudo-dyad includes inner floor of β -sheet 37-46, corner 47, outer cross-strand 47-51, return cross-strand 56-61 and outer floor 80-86. Back face dyad relates 27-35 to 91-99 at upper rear. Copper ligands are His 46, His 118, His 44, His 61. Zinc ligands are His 61, His 69, His 78, Asp 81.

partner across the dyad and rests against an extension of the opposite loop, residues 124-134. The local pseudo-dyad in the back face centred about strands *c* and *f* does not lie along the same line as the front dyad (see Fig. 2) and the two layers of β -sheet do not fit well to a single axis (see the third fit in Table 1).

A comparison of the amino acid sequences of the structurally equivalent regions³¹ also shows that the tripeptide Val-His-Gln at positions 45-47 is almost repeated across the dyad as Val-His-Glu at positions 117-119. The histidines are copper ligands, both valines are buried in the core of the barrel, while the glutamine and glutamic acid residues lie at sharp corners where the β -sheet leads into a cross-strand. There are no other sequence repeats associated with the repeated structure, although a few isolated equivalent residues match: Asp 50, Thr 56, Gly 83 to Asp 122, Thr 135, Gly 145 in the front face; Val 27, Ile 33 to Val 92, Ile 97 in the back face.

These comparisons show that the active site face of superoxide dismutase is formed by two similar paired subdomains which interlock symmetrically and enclose the catalytically essential copper ion at the centre of a shallow pit between two matching curved loops of irregular β -structure. Two of the histidine ligands occupy structurally equivalent positions in a repeated tripeptide near the dyad and 28 α -carbon atoms from each subdomain match within a root mean square distance of 1.58 Å. Approximately 14 of the related atom pairs lie in hydrogen-bonded regions of the β -sheet strands, but 14 others belong to the irregular encircling loops.

Since there is no internal homology in the amino acid sequence and only a limited structural regularity in the back face of the enzyme, all these repeated features associated with the active site might be the result of convergent evolution. However, the structural similarity between the dyad-related α -carbon backbones of the half-domains is relatively precise and compares favourably with the repeats in parvalbumin^{13,20} or pepsin^{14,15}. All the features may then be relics of a very early gene duplication and the enzyme could have evolved in the following way. (1) It began as a symmetrical dimer with an exact dyad axis, formed of two three-stranded β -sheet subdomains *c*, *d*, *e* and *f*, *g*, *h* which included the long *d*, *e* and *g*, *h* enveloping loops—compare the third fit in Table 1. (2) A copper ion was bound on the dyad to histidines 46 and 118, forming a primitive active site between the subunits. (3) A gene duplication united the halves of the dimer. (4) At some later time β -sheet strands *a*, *b* were added, completing the barrel and shifting the centre of the back face. (5) A zinc binding site developed at one side of the copper centre and the enveloping loops expanded between residues 61-80 and 123-135.

Although the structural evidence for this sequence of events is indirect every step has parallels in steps which have been proposed for the evolution of other symmetrical enzyme structures^{1,3,5}. The scheme would be consistent with the observation that the copper ion is essential for catalytic activity, while the zinc is not³² and can be replaced by other metals.

Richardson *et al.*²⁸ have pointed out a very interesting structural parallelism between the β -sheet barrels of superoxide dismutase and the immunoglobulin domains, which can be superposed with considerable precision. The immunoglobulins also have internal pseudo-dyad repeats (to be described elsewhere) which are considerably more regular than those in superoxide dismutase. Surprisingly the dyads do not match when the two proteins are superimposed. In superoxide dismutase the dyad passes between residues His 44 and Val 116 which lie on the outer surface of the Cu-Zn face. These correspond respectively to Tyr 42 and His 93 in the V_L domain²⁸ of McPC603. The dyad also passes between Gly 31 and Val 95 on the back face, which align with Met 21 and Leu 79 in V_L . But the best dyad for the immunoglobulin is displaced by one residue, passing between Trp 41 and Cys 94 on one face and between Ser 22 and Thr 78 on the other. This observation shows that it would be inconsistent to assume simultaneously that the two proteins have an evolutionary relationship and that both dyads also arose by gene duplication. Instead it seems probable that both barrels are independent realisations of a very restricted number³³ of allowed β -sheet topologies.

I thank Dr Jane Richardson for helpful discussions.

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BOOK REVIEWS

Worlds in collision

William M. Kaula

FOR about 20 years now, A.E. Ringwood has been the most vigorous propounder of theories in two areas: the state and evolution of the Earth's mantle, and the origin of the Earth and Moon. The book reviewed here is an exposition of his ideas on the second topic. Another, hefty book of Ringwood's, *Composition and Petrology of the Earth's Mantle* (McGraw-Hill: New York, 1975) deals with the first topic; the contents thereof are recapitulated in the first two chapters of *Origin of the Earth and Moon*.

Ringwood's scenario of Earth and Moon formation is the most complete extant, which makes it a convenient entity to quote, amend or attack. This relative completeness results not only from Ringwood's energy and breadth, but also from two other factors: firstly, the explanation of as many features as possible by processes in the interiors of the Earth or Moon, the regimes with which Ringwood is most familiar; and secondly, a strong philosophical drive to adopt the simplest of hypotheses wherever possible. Baldly, the essence of Ringwood's current scenario is:

(1) The Earth, like the other terrestrial planets, formed from solid matter which had condensed in the cooling solar nebula.

(2) This solid matter came to the Earth in the form of planetesimals, the composition of which evolved moderately so that their total bulk composition is 85% a component "A" of metals and silicates condensing at temperatures above $\sim 1200^\circ\text{C}$ and 15% a component "B" — "A" plus volatiles — principally alkali oxides, water, organics and sulphur compounds.

(3) Most of the volatiles were lost at planetesimal impact by being blown off by hydrogen liberated upon the interaction of iron and water.

(4) The iron core of the Earth formed early by the sinking of metal accumulations too large to attain chemical equilibrium with their silicate surroundings. One consequence was that appreciable traces of oxidized siderophiles (Ni, Co etc.) remained in the mantle.

(5) When core formation was essentially completed, a large body hit the Earth, vaporizing a lot of mantle material.

(6) An appreciable fraction of this

Origin of the Earth and Moon. By A.E. Ringwood. Pp.295. (Springer: Berlin, Heidelberg and New York, 1979.) DM45, \$24.80.

material was lifted into geocentric orbit, primarily by the energy of the impact, but possibly helped by interaction of ionized material with the early geomagnetic field.

(7) The refractory component of this cloud condensed, and accreted together to form the Moon.

Most of Ringwood's text is devoted to the geochemical (primary) and cosmochemical (secondary) considerations which lead him to this chain of hypotheses: such matters as the compositional differences of the Earth and chondritic meteorites; the lighter element admixed with iron to account for the core density; the reasons for chemical disequilibrium in the Earth; accounting for density differences among the terrestrial planets; the trace elements in lunar rocks compared to terrestrial rocks and meteorites; etc. He does not attempt to develop detailed physical models of the stages outlined above (anyone who does so conscientiously will have many years of work before he can write a book). Nonetheless, Ringwood's ideas are congenial to planetary physicists and dynamicists whose minds are unable to span the dizzying leap, from the nebula dust to the present bodies, required by those who hypothesize that compositional differences among planets arose from the same processes as compositional differences among meteorites.

The key evidence pertinent to lunar origin emphasized by Ringwood is the similarity of abundances of unvolatile siderophiles between the Moon and the Earth — with one exception (always exceptions in this game) within a factor of two among the elements examined. In contrast, there are appreciable depletions of more volatile siderophiles in the Moon compared to the Earth. These unvolatile siderophile abundances are much less than in chondritic meteorites, but much more than would occur in rocks crystallizing in equilibrium with metallic iron (which one category of differentiated meteorites, the eucrites, are believed to demonstrate). The great deficiency of iron in the Moon

required by its low mean density and absence of volatiles indicates prior planetary differentiation. Primordial isotope ratios, as well as dynamical feasibility, indicate that this differentiation was either in the Earth, or in other bodies at about the same distance from the Sun. The complete absence of any evidence of these other bodies in the meteorite population is the strongest argument in favour of the Earth for the locus of the differentiation.

The persuasive (although not overwhelming) lunar siderophile evidence comes from examination of low-titanium mare basalts, which are thought to be the consequence of rather high percentage partial melts deep within the Moon. More recently, Ringwood and collaborator John Delano have presented evidence of indigent siderophiles in lunar highland rocks and soils, an exercise requiring subtraction of a considerable meteoritic contamination, explanation of enrichment of the more volatile elements by "impact mobilization", etc. Here, Ringwood's drive to marshal all possible evidence in favour of a simple synthesizing hypothesis has again put him on the minority side of a rather technical debate. He was also, for example, about the last prominent petrologist to abandon the hypothesis that mare basalts were drawn entirely from source materials identical in composition with the bulk of the Moon.

Nonetheless, Ringwood's synthesizing drive, plus his physical intuition and sense of the issues, make this book the best introduction available to the main scientific debate generated by the Apollo project data, as well as a good target for his colleagues to snipe at. The reader coming from another scientific field will probably find much of the reasoning casual or *ad hoc*, the variety of data presented bewildering and the tone more one of strident advocacy than calm objectivity. These properties are not peculiar to Ringwood, but are rather characteristic of the field: strong intuitive biases are necessary to get anything done, and not be paralysed by indecision. The time for a Euclid is still far off. Meanwhile, a good book with much food for thought. □

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Of mercury and man

Roy Harrison

The Biogeochemistry of Mercury in the Environment. Edited by J.O. Nriagu. Pp. 696. (Elsevier/North-Holland: Amsterdam, Oxford and New York, 1979.) \$136.50, Dfl. 280.

THERE are few toxic substances with a history as long as that of mercury. The metal has been known and used for at least 3,500 years and still finds wide application today. There are documented instances of inorganic mercury poisoning dating back 500 years, and alkylmercury poisoning has been known for over 100 years. Yet mercury still provides surprises. Although the first cases of Minamata disease occurred in 1953, it was not until 1959 that alkylmercury poisoning was identified as the cause with any degree of certainty. Until that time, although occupational alkylmercury poisoning was known, the possibility of poisoning of the population by environmental exposure, in this case by

consumption of fish which had absorbed alkylmercury from the effluent of a local chemical works, had not been suspected. It was not known that fish could concentrate alkylmercury from water, nor was it discovered until later that alkylation of inorganic mercury in aquatic sediments was possible.

Minamata and other incidents of mercury poisoning have stimulated more interest in mercury as a pollutant than in any other toxic agent with the possible exception of lead. Biogeochemistry, a new term to me, apparently embraces "the chemical behaviour and biological effects of mercury in the biosphere, geosphere and hydrosphere". In Nriagu's own words, it provides "the logical conceptual framework for looking holistically at the mercury cycle and the human impact upon it". These are grand ideas and to a large measure the book lives up to them. The coverage is extremely broad, including the production, uses and control of mercury, the environmental chemistry and biological effects of the metal in terms of occupational and environmental human exposure, and the effects on aquatic and

terrestrial organisms. It includes detailed analyses of the outbreaks of organomercury poisoning at Minamata and Niigata in Japan (both due to consumption of contaminated fish) and in Iraq (due to the consumption of grain seed treated with mercurial fungicides).

The book contains 24 chapters, each written by specialists in a particular area. The result is quite impressive; there is a wealth of information which should satisfy most workers in this field, with relatively little overlap between contributions. Each chapter contains a large bibliography which is in most instances fairly up to date (references up to 1978 are included). As with much review material nowadays there is rarely any attempt at critical analysis, some chapters being little more than a listing of relevant work. That criticism aside, this book represents a valuable contribution for which many workers in this broad transdisciplinary field will be grateful. □

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Keeping up in physics

W. Cochran

A Perspective of Physics. Volume 3. Introduction by Sir Harrie Massey. Pp. 360. (Gordon and Breach: New York and London, 1979.) \$35.50.

IN 1695 delegates from the four Scottish Universities met as a Commission "to consider methods of instruction in philosophy". Their comments on draft reports covering various parts of the subject have been preserved, and we read for instance that "One part of the delegates are of opinion that Newton's hypothesis of the ebbing and flowing of the sea should be insert, or a reason given why it is not; and the other part think that there is no need to make any mention of it. The author gives this reason why he omitted it — because neither he nor any he has conversed with on the subject do so fully understand what Newton does write thereon as they can make it intelligible to young students". Any university teacher of physics who smiles at this in 1980 should do so wryly, for we have by no means solved by specialization that problem of keeping up to date with the advances made in our subject.

Volume 3 of *A Perspective of Physics* contains a selection of the articles which appeared in *Comments on Modern Physics* during 1978. This series appears in five sections, Nuclear and Particle Physics, Solid State Physics, Astrophysics, Atomic

and Molecular Physics, and Plasma Physics. In 1978 about 100 short review articles appeared under one or other of these headings, and of these 29 are reproduced in the volume under review. The emphasis is on brevity; a typical article is 3000–4000 words in length, with 20–30 references. Topics range from "Charge Density Maps in Chemisorption" to "New Quarks and Old Ones Too", and Sir Harrie Massey is remarkably successful in putting the articles into perspective in his introduction. I must admit, however, that

while I shall continue to read *Comments on Solid State Physics* as it appears, personally I find most other reviews in this series too terse and too specialist-directed when compared with those in, for example, *Physics Today*. We are better placed than our predecessors were three centuries ago, but there will never be an easy solution to the problem of understanding advances in physics. □

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A vitamin D encyclopaedia

D.R. Fraser

Vitamin D. The Calcium Homeostatic Steroid Hormone. By A.W. Norman. Pp. 496. (Academic: New York and London, 1979.) \$48, £27.

KNOWLEDGE about vitamin D, we are told, falls into eras. There was the era of ignorance before 1920, then the era of vitamin D chemistry and nutrition, followed by the 15–20-year era of vitamin D metabolism. We are now in the era of vitamin D reviews, with recent books entitled *Vitamin D* being produced by D.E.M. Lawson (Academic: New York and London, 1978), H.F. DeLuca (Springer: Heidelberg and New York, 1979) and this one by A.W. Norman, plus a myriad of review articles throughout the biological literature. Truly

the subject is well publicized.

One must admire the energy and enthusiasm resulting in this latest account. The work aims and succeeds at being comprehensive. There are detailed descriptions of the chemistry, metabolism and function of vitamin D, with chapters reviewing the historical development of the topic and the medical relevance of the new knowledge. All this is supported by an extensive bibliography, the dates in which indicate a minimum delay in the publication process. It is an admirable book of reference on all aspects of the biology of vitamin D.

On the other hand it is also the vitamin D gospel very much according to Norman, and hence tends to be a catalogue of the notable achievements of the author and his colleagues. But in a curious way an opportunity has been missed here to give a lead in unravelling the complexities of vitamin D function. Rarely does the author allow his own insight to throw new light on this problem. Yet the mysteries of the mechanism of action of vitamin D are now more amen-

able to attack by imaginative intellect than they ever were in the experiments of the past.

Obviously with just one author, the significance of each finding receives very personal assessment. One might wish that more emphasis had been placed on the discovery of the 25-hydroxylation reaction which was the key finding in the identification of functional vitamin D metabolism. There is also a pervasive, conscious attribution of discovery to discoverer to the point of obsession. After noting the seventeenth century descriptions of rickets by

Whistler and by Glisson, it was concluded "only fair and reasonable to give both authors credit for first priority". With the passage of time do scientists really care who did what first?

It is helpful to have the author's special knowledge of such topics as the relationship between structure and biological activity of vitamin D and the use of filipin in studies on calcium transport. It is possible to ignore the smattering of errors which mar the text but perhaps it would be wise to correct the bizarre footnote on

afatoxin (p.446) in any subsequent edition. There are many attractive figures and tables to illustrate the work; in some of these their artistry exceeds their comprehensibility (e.g. Fig. 6-22).

Using some of the author's favourite words, this book could be described as an exceedingly potent, dramatic, and unequivocally massive, herculean effort. It's also quite a good read. □

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Nodules in depth

Roger G. Burns

Manganese Nodules. By R. Sorensen and R. Fewkes. Pp.723. (IFI/Plenum: New York and London, 1979.) £59.85.

MANGANESE nodules are intriguing marine deposits because contained in the very fine-grained matrix are accumulations of the strategic metals cobalt, nickel, copper, manganese and molybdenum. Understanding how these deep-sea ferromanganese oxide concretions formed, and the processes by which the transition metals became enriched in the constituent manganese and iron oxide phases, requires access to very high resolution observational and analytical instrumentation. Sorensen and Fewkes have catalogued data compiled at Washington State University during the past decade for several hundred marine manganese nodules using techniques of ore microscopy, X-ray diffraction, X-ray microprobe and electron microprobe analyses. Almost half of their book consists of photographs of polished sections of manganese nodules collected from the Pacific Ocean as well as some specimens from the Atlantic and Indian Oceans. The remainder of the book documents data and describes methods used to study each of the nodules.

Five introductory chapters in Part 1 of the book (142 pages) provide detailed accounts of procedures to prepare polished mounts of manganese nodules and to photograph them in reflected light. Techniques are described for obtaining microsamples for mineral identification by X-ray diffraction, and analysing the nodules non-destructively by X-ray microprobe and electron microprobe methods. Results of studies of internal textures, mineralogy and probe analyses of some of the nodules are given and attempts made to interpret the data. The text reads like a self-centred report to a funding agency; reference to pertinent work of other investigators is minimal.

The second part of the book (581 pages) is an encyclopaedia of data sheets and photomicrographs of almost 300 nodules

studied. The reflected light photographs of the polished sections are superb; they provide a plethora of information on the diverse internal structures and growth histories of the nodules. The data sheets painstakingly summarize the seafloor locations, physical properties and available laboratory data for each of the nodules. The features soon become repetitive, however, contradicting the authors' declared major purpose of the volume, which is "to provide the interested investigator with a factual record of the internal features of typical nodules".

Most of the information contained in the book is now well known to scientists studying manganese nodule geochemistry. One might question whether the frequency of occurrence of birnessite reported by the authors alone is an artifact of conditions (air drying; vacuum impregnation) used to prepare and mount their manganese nodule specimens. Current investigations indicate that delta-MnO₂ (vernadite) is as abundant as todorokite, the major host of nickel and copper, in Pacific nodules. Some of the observations and interpretations made by the authors have been superseded by results from more modern high-resolution techniques, including scanning electron microscopy and energy dispersive analysis, transmission electron microscopy and selected area diffraction, infrared and extended X-ray absorption fine structure spectroscopy. The authors do not refer to the wealth of information on synthetic mineral analogues and bacteriological studies relating to the stability and formation of manganese oxide phases hosting transition metals in manganese nodules, which drastically modify internal structures and microchemistries of manganese nodules.

The prohibitive price means that it is unlikely that individuals will buy this book. However, the magnificent photographs make the volume a showpiece for coffee tables and library display shelves. □

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Two on physical and theoretical chemistry

J.S. Rowlinson

Statistical Thermodynamics of Simple Liquids and their Mixtures. By T. Boublik, I. Nezbeda and K. Hlavaty. Pp.145. (Elsevier: Amsterdam and New York, 1980.) \$41.50, Dfl.85. **Weak Intermolecular Interactions in Chemistry and Biology.** By P. Hobza and R. Zahradnik. Pp.346. (Elsevier: Amsterdam and New York, 1980.) \$48.75, Dfl.100.

THESE two monographs are the second and third in a series called "Studies in Physical and Theoretical Chemistry". Both are by Czech authors, and both are out-of-date, but there the resemblance ends. The book by Boublik and his colleagues is a compact and balanced account of the statistical mechanics of model liquids composed of spherical particles. There is a short chapter on integral equations for the molecular distribution functions and two long chapters on perturbation theory. The treatment is good on the mathematical details but cursory on the comparison of theory with the results of computer simulation, while real liquids receive but the barest mention. There are only two references later than 1974 (both Czech) and so the book is silent on the most rapidly growing part of the field in the last ten years — the extension of theories to deal with non-spherical molecules. Within these limitations it can be recommended to those who want a clear account of the authors' chosen field, and are prepared to pay the stiff price.

Weak intermolecular interactions is, perhaps, a more challenging subject since so many techniques contribute to its study: spectroscopy of many different kinds, molecular beam scattering, thermodynamic and transport properties, quantum mechanics and both equilibrium and non-equilibrium statistical mechanics. It is a challenge that this badly balanced

book quite fails to meet. It starts with a pedestrian but well-referenced chapter on quantal calculations, which omits the estimation of dispersion forces from transition moments, and overlooks the existence of multibody forces in condensed systems. It starts to go badly wrong when it gets to experimental methods and their interpretation. Inversion of macroscopic properties to give intermolecular potentials

is wrongly described as "rarely successful", electronic spectroscopy of dimers is dismissed in seven lines, and molecular beams in two pages. The section on the determination of intermolecular forces from viscosity is so misjudged that it quotes equations appropriate to the theory of the viscosity of liquids whilst only gases have been used for this purpose. The final chapter, "Applications", has some more

interesting pages but it is often uncritical and repetitious. Even the useful information in the book is difficult to find without an index of systems. This disappointing book can be recommended neither to chemists nor to biologists. □

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Thirst work

M.J. McKinley
and J.F. Nelson

The Physiology of Thirst and Sodium Appetite. By J.T. Fitzsimons. Pp.572. (Cambridge University Press: Cambridge, UK, 1979.) £32.50.

RENEWED interest in the study of thirst mechanisms has resulted from the experimental contributions made over the past two decades by James Fitzsimons. The last major work on this subject, *Thirst* by A.V. Wolf (Charles C. Thomas: Springfield,

Illinois, 1958), appeared more than 20 years ago and, in view of the burgeoning experimental activity in this field since then, Fitzsimons' new book is a timely and comprehensive account of knowledge on thirst.

His book is the latest of the series of monographs published by the Physiological Society. Designed primarily for readers at the level of graduate student and above, it gives a lucid analysis of the causes of drinking, discusses the comparative physiology of drinking in vertebrates, dissects the effect of cellular and extracellular dehydration, and assesses the roles of hormones and the renin-angiotensin system on drinking behaviour. A survey of the pharmacology of drinking

and clinical aspects of thirst is included as well as a chapter on sodium appetite.

It is perhaps an unfortunate necessity that, as recognized by the author, the book is no more than an interim account. Thus few of the more than 700 references cited are to results published by others during the last three years. As a consequence, for example, the importance placed by him on the renin-angiotensin system as a stimulus to thirst would now be disputed by a number of other investigators in the field.

The chapter on sodium appetite has a number of deficiencies. This is primarily a consequence of defining sodium appetite as "an innate response to sodium deficiency, that causes animals to seek and ingest sodium salts", a restrictive definition. Thus evidence which demonstrates clearly that glucocorticoids stimulate sodium appetite has been overlooked. In fact the author claims that "glucocorticoids have little if any action on sodium appetite". It also appears to be inconsistent with such a definition that he recognizes that sodium appetite increases in both rats and rabbits during pregnancy and lactation where the changes are almost certainly due to alterations in hormone secretion and not to sodium deficiency.

The synthesis of the large salt appetite of pregnancy and lactation by appropriate sequential administration of physiological amounts of steroid and peptide hormones has been shown. The experiments closely parallel those on induction of maternal behaviour by similar hormone dosage. They hardly leave any conclusions on the role of these hormones as hazardous, as the author suggests, unless quite different criteria are applied to those advanced by him for a physiological role of angiotensin in thirst. Some minor inconsistencies and errors (e.g. p.100 lists plasma K of humans as 6.3mM) in the text were also noted.

Despite these limitations the book makes a very significant contribution to the dissemination of knowledge in this rapidly advancing area of physiology, and will no doubt become a standard reference work on thirst. □

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Hagfishes and lampreys

Lis Olesen Larsen

Biology of the Cyclostomes. M.W. Hardisty. Pp.428. (Chapman and Hall: London, 1979.) £20.

THOSE who wish to elucidate the early stages of vertebrate phylogeny often examine only one, or at most two, cyclostome species. Further, such studies have too often been based on a few animals, badly described as to prehistory, stage of development, sex etc. This makes much of the ample literature on cyclostomes (hagfishes and lampreys) rather superficial. Professor Hardisty, however, has spent a lifetime in devoted study of many aspects of lamprey ecology, physiology and morphology. Recently retired, he has made in this book an impressive effort to summarize what is known about lampreys and hagfishes. His main intention is to discuss the early stages of vertebrate phylogeny by comparing all aspects of the two groups. His basic assumption is that although cyclostomes are no longer considered directly ancestral to the gnathostomes, they are more likely to have retained characters of "the unknown vertebrate ancestor" than other vertebrate groups.

Each chapter compares lampreys and hagfishes, and the topics range from palaeontology, ecology, physiology and

morphology, to biochemistry and molecular biology. In a final chapter Hardisty tries to draw conclusions about evolution on the basis of an integrated view of the life of hagfishes and lampreys. Thanks to his honest discussions, this chapter made it clear to me (but not to him) that his basic assumption is not necessarily true. The probability of finding primitive characters in present-day vertebrates may be equally large in any vertebrate group. In addition our hypotheses about evolutionary pathways are always marred by the difficulties in distinguishing between primitive and specialized characters, and in knowing when changes are adaptive (that is caused by natural selection) and when they occur by chance. The latter possibility is not seriously considered by Hardisty.

Throughout the book the author lists differences, between lampreys and hagfishes and between cyclostomes and gnathostomes, in attempts to establish how these three groups relate to one another. In order to obtain knowledge about the vertebrate ancestor(s!) it may be equally useful to look for similarities among these three groups, and between each of them and the various invertebrate and protochordate groups.

The book is recommended for all who want to study cyclostomes. They will find a useful, broad and detailed basis for their studies and an updated list of references. □

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